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SIGNIFICANCE OF PALPABLE SPLEENS IN MALARIOUS AREAS OF LOW ENDEMICITY*

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The association of splenomegaly and malaria has long been noted, and in the areas free of kala azar and schistosomiasis the number of people exhibiting enlarged spleens has been considered roughly proportional to the number having malaria. A large body of evidence supporting this view has been recorded for areas of high endemicity. It is not the purpose of this paper to review the evidence but there appears to be general agreement on this point.

In areas of low endemicity, however, the prevalence of splenomegaly established by examination techniques designed to elicit only readily palpable spleens, has been found too small to be of practical significance (Maxcy, 1925; Maxcy, et al, 1927). To increase the usefulness of spleen surveys, Darling (1924, 1925, 1926) suggested that the technique should be made as delicate as possible to determine the least degree of splenic enlargement. His technique introduced the term of "spleen just palpable on deep inspiration" (PDI) and in consequence increased considerably the proportion of children exhibiting palpable spleens.

These barely palpable spleens are thought by many to be just as significant in the measurement of malaria as those palpable at or below the costal margin on normal inspiration (Darling, 1924, 1925, 1926 Hackett, 1944; Russell, 1935). Other workers have raised doubt as to the validity of this position when the more delicate technique of palpation is applied in areas of low endemicity (Carley, et al, 1929; Cantrill 1942; Maxcy et al 1927)

In view of this divergence of opinion, before using the method on a large scale in an effort to delineate foci of malaria, it was deemed desirable to compare examinations made in an area believed to be malarious with similar examinations made in a non-malarious area. Arrangements were therefore made to compare a group of children in a known non-malarious section with a group in a reputedly malarious section of the South. The survey was carried out in the spring of 1944: March in Arkansas, April in Massachusetts, and in New York during April and May.

Phillips and Jefferson Counties, Arkansas, were chosen to rep-

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resent typical sections where malaria surveys were indicated. They were selected on the basis of death certificates for malaria over the five year period 1938-1942. For this period Jefferson County, with an average annual death rate of 22.7 ranked twenty-seventh among the counties of thirteen southeastern states in deaths per 100,000 population and ninth in deaths per 1,000 square miles (16.6). Phillips County was thirty-second in deaths per 100,000 (21.8) and eighteenth in deaths per 1,000 square miles (14.2).

The areas chosen to represent non-malarious sections were Fulton and Montgomery Counties and Harlem and New York City in New York and communities in the vicinity of Boston and New Bedford, Massachusetts. Malaria has not existed in endemic form in these localities for at least twenty years.

A blood slide survey was not done in conjunction with the spleen survey because recent blood surveys have shown a very low percentage of positives throughout reputedly malarious areas of the South. Thick film surveys carried out in schools during the fall of 1943 resulted in only one positive smear for malaria out of 1,985 examined in Jefferson County, and no positives in Phillips County where 1,171 slides were taken for examination. It is known that blood slide surveys give a lower index of malaria than the actual amount present (Hackett, 1944) and this is especially true in the South where the use of chill tonic is very widespread.

All children were examined in the recumbent position with thighs and knees flexed and abdomen bared sufficiently to allow palpation of the left upper quadrant. The examiner stood at the right of the patient and palpated lightly with the right hand held flatly on the abdomen more or less at right angles to the costal margin. If the spleen was not palpated on normal inspiration the child was instructed to breathe deeply. Every effort was made to avoid exciting the child so that the fullest relaxation could be obtained. All examinations were done by the writer to assure uniformity of findings. The writer had a period of training in Florida during the fall of 1943 under Dr. John E. Elmendorf, Jr. of the Rockefeller Foundation. Over 6,000 children were examined during that season.

Results were recorded according to the classification of Boyd (1929) which divides palpable spleens into five groups.

If the border of the spleen was palpated at the coastal margin on deep inspiration it was recorded as a PDI (palpable on deep inspiration only). If it was palpable at the costal margin (on normal inspiration) and extended no farther below the costal margin than one centimeter on deep inspiration it was recorded as number one. A spleen palpated from this point to a position half way to the umbilicus along a line drawn from the right anterior superior iliac spine

through the umbilicus to the curve of the left costal margin was recorded as number two. A spleen palpated farther along this line to the umbilicus on deep inspiration was classed as number three. Any extending beyond the umbilicus was recorded as number four. The examination was limited to children between the ages of five and twelve as much as possible since spleen surveys usually include this group. Usually about two hundred children were examined in a day. Other physicians were present much of the time and were usually able to corroborate the writer's findings after a little practice. If a child was too uncooperative to permit a satisfactory examination the result was recorded as unsatisfactory and not included in the analyses. Only 80 (1.2 per cent) of the 6,549 children examined were so recorded.

In analyzing the results of the survey many different comparisons are made. White children are separated from negro children in the analyses because spleen rates differ in the two races (Boyd, 1929, 1930; Carley et al 1929; Carr & Hill 1942; Clark, 1928; Gray 1942). Tables which appear below include PDI spleens in one group and all larger spleens in another. There were only eleven spleens of class number two, six of class number three and none of class number four. There were only 13 children of races other than white or negro.

Comparison of White and Negro Children Exhibiting Palpable Spleens

Table 1 gives a comparison of the proportion of white and negro children exhibiting palpable spleens. It is seen that the prevalence of such spleens is much less in negro than in white children and that this phenomonon is true for the spleens palpable only on deep inspiration as well as the larger spleens. As pointed out in the preceding paragraph the lower prevalence of palpable spleens in negro children is an established fact and is probably a racial characteristic.

TABLE 1
Comparison of Palpable Spleens in White and Negro Children

Race	Number of Children Examined	*Children Exhibiting Palpable Spleens*				
		Number		*Percent*		
		PDI	Larger than PDI	PDI	Larger than PDI	All Classes
White	4571	510	243	11.2	5.3	16.5
Negro	1885	83	20	4.4	1.1	5.5
Total	6456	593	263	9.2	4.1	13.3

Comparison of Negro Children of Malarious and Non-malarious Areas Exhibiting Palpable Spleens

Table 2 compares the prevalence of palpable spleens in negro

children of malarious and non-malarious areas. The difference in percentages shown are not large enough to be of value in estimating malariousness. A difference as small as that found between palbable spleen of all classes in negro children of the North and South, 4.9 per cent to 6.3 per cent, might occur by chance in seventeen out of one hundred such samples. This finding is important because it indicates that negro children who comprise a large proportion of the susceptible population of most Southern communities should not be included in spleen surveys when endemicity is low.

TABLE 2
Negro Children Exhibiting Palpable Spleens in Non-malarious Areas and Areas of Low Malaria Endemicity

Type of Area	Number of Children Examined	*Children Exhibiting Palpable Spleens*				
		Number		Percent		
		PDI	Larger than PDI	PDI	Larger than PDI	All Classes
Malarious	775	41	8	5.3	1.0	6.3
Non-Malarious	1110	42	12	3.8	1.1	4.9
Total	1885	83	20	4.4	1.1	5.5

Comparison of White Children Exhibiting Palpable Spleens in Malarious Areas and Areas of Low Malaria Endemicity

Table 3 gives the same comparison for white children as shown in Table 2 for negro children. There is seen in contrast to negro children a significantly higher percentage of palpable spleens in Southern white children than in Northern white children. This difference is due almost entirely to the spleens larger than those palpable only on deep inspiration. There is no significant difference in the percentages of PDI spleens of the two areas, 12.2 per cent and 10.8 per cent, the probability being seventeen in one hundred this difference could be due to chance. Some encouragement is offered by these findings for the use of spleen surveys among white children an as index of malaria in areas of low endemicity provided PDI spleen be disregarded.

TABLE 3
White Children Exhibiting Palpable Spleens in Non-malarious Areas and Areas of Low Malaria Endemicity

Type of Area	Number of Children Examined	*Number*		*Percent*		
		PDI	Larger than PDI	PDI	Larger than PDI	All Classes
Malarious	1219	149	135	12.2	11.1	23.3
Non-Malarious	3352	361	108	10.8	3.2	14.0
Total	4571	510	243	11.2	5.3	16.5

Effect of Recent Illness on Prevalence of Palpable Spleens

In the foregoing tables no attempt has been made to exclude children moving into their community recently or having any illness other than malaria which might effect the spleen size Ordi-

narily in surveys for malaria all children are included as they are in these tables. A study of the nature of this paper requires more detailed analyses.

Table 4 compares the percentage of palpable spleens in white children giving a history of recent illness to that of children giving no such history. The only illness recorded are those causing continuous absence of a week or more in the three months preceding the examination. Inspection of this table indicates that those having a recent illness present a higher percentage of palpable spleens than those not ill. These differences are significant in the children of malarious as well as non-malarious areas. A difference of the order exhibited in Southern children, 29.6 per cent against 21.2 per cent, might occur by chance in only three out of one thousand similar samples. It is interesting to note that the children of malarious areas present the greatest difference. One wonders if perhaps a spleen is rendered more sensitive to other diseases by a previous attack of malaria.

TABLE 4
White Children: Effect of History of Recent Illness

	Malarious Area				*Non-Malarious Area*			
		Larger than %		Spleens		Larger than %		Spleens
Diseases	Cases	PDI	PDI	Palpable	Cases	PDI	PDI	Palpable
Measles	153	23	22	29.4	8	1	0	12.5
Chicken Pox	13	2	3	38.5	150	16	8	16.0
Flu	60	7	10	28.3	48	3	4	14.6
Colds	4	0	0	0.0	222	28	11	17.6
Others	74	13	10	31.1	244	33	7	16.4
Total Ill	304	45	45	29.6	672	81	30	16.5
No Illness	915	104	90	21.2	2680	280	78	13.4

On further inspection of Table 4 it is seen that the increased incidence of palpable spleens is exhibited with practically all the diseases listed; the ones not showing the increase comprise too small a sample for comparison. Some of the diseases included in the classification "other diseases" are whooping cough, mumps, tonsilitis, pneumonia, sore throat, "swollen glands," impetigo, bronchitis, bronchiectasis, pink eye, scabies, kidney trouble, asthma, otitis media, and stomach trouble. None of them constitue a large enough group for comparison. An interesting observation is that only four Southern children are recorded as having malaria.

Table 5 shows the relationship of illness to palpable spleens in negro children. As is the case with white children a higher percentage is present in the colored group giving a history of recent illness, but the differences are not significant. Measles is the only disease which appreciably affects the rates.

TABLE 5
Negro Children: Effect of History of Recent Illness

Disease	*Non-Malarious Area*				*Malarious Area*			
	Cases	PDI	Larger than PDI	% Spleens Palpable	Cases	PDI	Larger than PDI	%Spleens Palpable
Measles	100	11	2	13.0	39	3	2	12.8
Chicken Pox	3	0	0	0.0	45	3	0	6.7
Flu	7	0	0	0.0	2	0	0	0.0
Colds	9	0	0	0.0	40	1	0	2.5
Others	33	0	0	0.0	58	1	2	5.2
Total Ill	152	11	2	8.6	184	8	4	6.5
No Illness	623	30	6	5.8	892	33	8	4.6

There is no significant difference between the spleen rates and the group moving within the year. This is probably due to the fact that Southern children usually moved in from a similar Southern community and new arrivals in Northern communities were usually from the North.

Comparison of Palpable Spleen of Children in Malarious and Non-malarious Areas Excluding those with Recent Illness and Moving in within the Year.

In Table 6 white children are excluded who either moved into their county within the year or were ill within the preceding three months. The same relationship is shown between children living in malarious and non-malarious areas that was indicated in Table 2 when no one is excluded, namely that there is a higher percentage of palpable spleens in southern than northern children and that this difference is found chiefly in the class of spleens larger than PDI.

TABLE 6
White Children Not Recently Ill, Resident in their County One Year or More. Comparison Non-malarious Areas and Areas of Low Malaria Endemicity.

Type of Area	Number of Children Examined	*Children Exhibiting Palpable Spleens* — *Number* PDI	Larger than PDI	*Per Cent* PDI	Larger than PDI	All Classes
Malarious	777	94	76	12.1	9.8	21.9
Non-Malarious	2596	274	77	10.6	3.0	13.5
Total	3373	368	153	10.9	4.5	15.4

Table 7 gives the same relationship for Negro children that Table 6 shows for white children. As indicated previously in Table 3 no significant difference is seen between spleen rates of Southern and Northern negroes, the prevalence being even more nearly comparable in Table 7.

TABLE 7

Negro Children Not Recently Ill, Resident in their County One Year or More. Comparison Non-malarious Areas and Areas of Low Malaria Endemicity.

Type of Area	Number of Children Examined	*Children Exhibiting Palpable Spleens*				
		Number		*Per Cent*		
		PDI	Larger than PDI	PDI	Larger than PDI	All Classes
Malarious	607	26	6	4.3	1.0	5.3
Non-Malarious	892	33	8	3.7	0.9	4.6
Total	1499	59	14	3.9	0.9	4.9

Comparison of Palpable Spleens in Children of New York and Massachusetts

An interesting comparison is that of the white children of New York to those of Massachusetts shown in Table 8. There is a significantly higher percentage exhibiting spleens larger than PDI in New York, but no significant difference in those having PDI spleens. A difference of the order shown here, 3.7 per cent of 1917 New York children and 679 in Massachusetts with 1.0 per cent would occur by chance in five out of ten thousand similar samples. An obvious explanation of this fact cannot be offered as malaria is non-existent in both areas and the samples exclude all children who are new arrivals or had a recent illness.

Negro children of New York and Massachusetts show a similar relationship. None of the one hundred negro children examined in Boston had palpable spleens while palpable spleens were found in 5.0 per cent of 824 children in New York.

TABLE 8

White Children, Resident One Year of More in County, Not Recently Ill. New York and Massachusetts

Area	Number of Children Examined	*Children Exhibiting Palpable Spleens*				
		Number		*Per Cent*		
		PDI	Larger than PDI	PDI	Larger than PDI	All Classes
New York	1917	203	70	10.6	3.7	14.2
Massachusetts	670	71	7	10.5	1.0	11.5
Total	2596	274	77	10.6	3.0	13.5

Relation of Palpable Spleens to Age

Table 9 shows the percentages of palpable spleens in children of the age groups 4 through 9 and 10 and over. The younger age group has a slightly greater prevalence in practically every comparison, but in only one case is the difference statistically significant. This is in the negro children of malarious areas exhibiting PDI spleens, 7.2 per cent of 389 younger children and 3.4 per cent of

386 older children having spleens of this size. A difference of this order might occur by chance alone in 17 out of 1,000 similar samples.

TABLE 9
Relation of Palpable Spleens to Age

Race	Type of Area	Age Group	Number of Children	Number PDI	Number Larger than PDI	Percent PDI	Percent Larger than PDI	All Classes
White	Malarious	4 - 9	760	92	94	12.1	12.4	24.5
		10 & +	459	57	41	12.4	8.9	21.4
	Non-Malarious	4 - 9	1659	191	62	11.5	3.7	15.3
		10 & +	1693	170	46	10.0	2.7	12.8
Negro	Malarious	4 - 9	389	28	4	7.2	1.0	8.2
		10 & +	386	13	4	3.4	1.0	4.4
	Non-Malarious	4 - 9	575	26	10	4.5	1.7	6.3
		10 & +	535	16	2	3.0	0.4	3.4

Relation of Palpable Spleens to Sex

A comparison of palpable spleens in boys and girls is shown in Table 10. It is seen that males show a greater prevalence of palpable spleens than females and that the difference is found only in the class of spleen palpable on deep inspiration. Only two of these differences are statistically significant; namely white children of malarious areas and negro children of non-malarious areas. Differences shown in these two comparisons might occur by chance six times out of one thousand in the first case and thirty-eight out of one thousand in the second.

TABLE 10
Relation of Palpable Spleens to Sex

Type of Area	Sex	Number of Children	*Children Exhibiting Palpable Spleens* — *Number* PDI	*Number* Larger than PDI	*Per Cent* PDI	*Per Cent* Larger than PDI	All Classes
White Malarious	M	607	90	67	14.8	11.0	25.9
	F	612	59	68	9.6	11.1	20.8
Negro Malarious	M	1672	197	54	11.8	3.2	15.0
	F	1680	164	54	9.8	3.2	13.0
Non-Malarious	M	385	26	3	6.8	0.8	7.5
	F	390	15	5	3.8	1.3	5.1
Non-Malarious	F	463	24	5	5.2	1.1	6.3
	M	647	18	7	2.8	1.1	3.9

Summary and Conclusions

To summarize, the analysis of the survey results shows a large percentage of palpable spleens among white children in areas of low malaria endemicity than in children living in non-malarious areas. This difference is found only in children with spleens larger than those palpable on deep inspiration while the percentage of spleens palpable only on deep inspiration is practically the same in both areas. Negro children have a lower incidence of palable spleens

than white children. There is no significant difference between the prevalence of palpable spleens in negro children of the North compared to those of the South. The percentage of white children exhibiting spleens larger than palpable on deep inspiration is significantly greater in children of New York than in those of Massachusetts. Children with a history of recent illness have a higher incidence of palpable spleens than children giving no such history.

In view of these findings one must be careful when attempting to use the result of a spleen survey to estimate the malariousness of an area of low endemicity. The percentage of spleens palpable only on deep inspiration is of little or no significance since a high prevalence is found among apparently normal children of non-malarious areas. The number of children exhibiting spleens larger than those palpable on deep inspiration may be of some assistance in arriving at an estimation. One must be careful to discount the possibility of an increased number of palpable spleens due to recent illnesses among the children of a locality. There may be other unknown factors affecting the prevalence of palpable spleens as indicated by the higher percentage of spleen larger than palpable on deep inspiration in white children of New York compared to those of Massachusetts. An important observation is that negro children who comprise a large proportion of the population of Southern communities apparently must be eliminated from spleen surveys in areas of low malaria endemicity. Age and sex play minor roles in the prevalence of palpable spleens among children. As the incidence of malaria falls in any locality the spleen rate becomes lower until a point is reached below which the spleen rate can no longer be used as an accurate measure of malaria.

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School surveys were done in Boston, Quincy, Acushnet, Dartmouth and New Bedford in Massachusetts. In New York surveys were done in the Harlem area of New York City, Amsterdam, Gloversville, Johnston, Northville and Canajoharia. In Arkansas surveys were done in Pine Bluff, Helena and surrounding rural schools.

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THE SPLEEN SURVEY AS APPLIED TO THE MEASURE OF MALARIA IN THE UNITED STATES – A REVIEW OF THE LITERATURE

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There is some controversy as to the relative merits of the two practicable, objective methods for determining malaria prevalence. The thick blood film survey has certain advantages and so has the spleen index. In the summer of 1942, the Malaria Control in War Areas office of the United States Public Health Service in conjunction with state health departments conducted a blood slide survey, collecting 100,000 thick films throughout the endemic areas of this country. Less than 0.2 per cent of these were positive, and nowhere was the number of positives high enough for the method to be useful as a means either of checking the effect of malaria control projects or of indicating need for additional projects. On the other hand, Dr. J. E. Elmendorf used spleen surveys at the same time in Florida and found 20 to 25 per cent of school children to have palpable spleen, despite the fact that less than 1 per cent had a positive blood slide. Partly to seek explanation of this situation and partly to evaluate the status of splenometry under the hypodemic conditions of the United States, recourse was taken to the literature. The following review attempts to determine the specificity of the spleen index and the significance of difficultly palpable spleens. It also is concerned with the relationship between spleen and parasite indices and with factors affecting the spleen reaction.

Differentiation of Splenic Enlargement not due to Malaria

Dempster was the first to observe the frequent occurrence of splenomegaly in fever infested areas; and Ross (1910) later developed the use of splenometry as an index to the amount of malaria. He and many others subsequently concerned have satisfied themselves that the spleen survey can be sufficiently specific for malaria. Where malaria is obvious the spleen survey gives an accurate measure of the disease. Kala-azar and schistosomiasis are the only other infections needing differentation. Kala-azar is important in Southern Spain, Italy and North Africa and in parts of India, China, and Russia; schistosomiasis is important in regions of Africa, Tropical America and China. An idiopathic enlargement of the spleen, termed "Bengal Splenomegaly," by some believed due to malnutrition, is said to require differentation from splenomegaly of malaria in India (De 1939). In the United States

where malarial infection is slight the value of spleen examination is debatable. Here the season of transmission is short, and accordingly malaria endemicity is relatively low, and the "average enlarged spleen" is small (Hackett, 1944). There are several conditions other than malaria which have been considered as causing a significant number of enlarged spleens in surveys here. Fort (1926) found 75 per cent of school children in presumably nonmalarious Dawson, Georgia to have palpable spleens which he could differentiate clinically from those of malaria, and which he attributed to a recent epidemic of measles in the community. Thirty days after the first examination 85 per cent of these were palpable, and after six months he still could palpate 20 per cent of them. He found also a palpable spleen in 5 per cent of children with chronic tonsillitis. Examining 397 children with measles, Bleyer (1926) observed a spleen index of approximately 50 per cent on the third day of the rash but of less than 15 percent after two weeks. Boyd (1930) provides evidence to show that splenomegaly in measles and chicken pox is transitory; he expresses the opinion that an acutely enlarged spleen subsides rapidly after a cure whereas one chronically stimulated to enlargement persists for a considerable time. Rare metabolic and hematologic causes of splenomegaly do not significantly affect spleen rates (Boyd, 1930). Russell (1935) mentions that, in Massachusetts, children with a history of scarlet fever had a relatively high index of spleens palpable on deep inspiration. An indication of the proportion with spleen enlargement in acute illness is to be found in Table I from Bleyer (1927).

TABLE 1

Spleen Examinations of 1,000 Acutely Ill Children.

Diseases	*No. Examined*	*Per Cent Positive Spleens*
Diphtheria	67	13
Scarlet Fever	167	17
Measles	120	47
Rubella	27	10
Pneumonia	36	25
Whooping Cough	57	18
Smallpox	2	0
Chicken Pox	37	38
Active Lues	28	98
Inactive Lues	15	8
Otitis	47	8
Tonsillitis	33	21
Very active rickets	140	35
Malnutrition	111	13
Scurvy	2	0
Eczema	37	19
Impetigo	19	11
Ulcerative stomatitis	12	23
Mongolism	14	14

Rickets, it was observed, resulted in splenomegaly only when

very active. Malnutrition in these children was said not to be associated with enlargement of the spleen. Russell (1935) observed a palpable spleen rate of 5.7 per cent in 156 cases of advanced childhood type tuberculosis and a rate of 0.0 in 55 minimal or moderately advanced cases. A possible relationship between enlargement of the spleen and both hemoglobin level and enlargement of the liver has been suggested by Zamkin (1925). A statistical study of 1,028 infants and children with splenomegaly in a Chinese clinic revealed that enlargement occurs most readily in infants under two years of age and that it may most frequently be attributed to infections of the upper or lower respiratory tract (Chu et al, 1939).

It appears then that a recent epidemic of one of the childhood diseases (particularly measles) or perhaps of respiratory illness and a high rate of chronic tonsillitis are the conditions which may interfere with school spleen surveys in the United States.

How often normal spleens are palpable needs of course to be determined. Divergent estimates have been made, based on surveys of school children in non-malarious areas. The results of such studies, depend very largely upon the technique employed; for there are a number of normal persons in whom an experienced examiner can palpate the spleen at the end of a deep inspiration. The significance of P. D. I. (palpable on deep inspiration) spleens as regards malaria will be considered later, but it may be emphasized here that the results will only be reliable after the examiner has standardized his technique both by examining a number of children in malaria free areas and by checking his results against those of others expert in the technique. Boyd has described the proper technique of palpation and has given a classification of spleen sizes that has been widely employed (Boyd, 1930). Hackett has recently modified this (Hackett, 1944).

A study by Zamkin on normal infants and children presenting themselves at New York clinics revealed the spleen rates given in Table 2, (Zamkin, 1925). Children with a history suspicious of syphilis, malaria, blood dyscrasia, jaundice, rickets or chronic infection, were excluded from this series; those acutely ill were temporarily withheld; those with hemoglobin below 75 per cent or

TABLE 2

Spleen Examinations of Active New York City Children

Ages Examined	*No. Examined*	*No. Spleen Positive*	*Per Cent Spleen Positive*
10 days to 1 yr.	541	233	43.1
2 to 4 yrs.	458	138	30.0
5 to 9 yrs.	835	153	18.3
10 to 12 yrs.	266	29	10.9

with positive serology were excluded.

This bears out the generally accepted theory that the spleen is frequently palpable in infants. A summary of several published surveys of non-malarious areas, as presented in Table 3, seems to indicate that those experienced in malaria splenometry and familiar with the sensitive method of spleen examination find less than 15 per cent of palpable spleens in school children. These may be caused by extreme extension of the spleen pedicle, depression of the diaphragmatic arch, abnormal thoracic structure, or recent illness.

TABLE 3

Spleen Examinations on Children in Non-malarious Areas including Spleens Palpable on Deep Inspiration

Author and Reference	*Place*	*Number Examined*	*Race*	*Per Cent Palpable*	*Pct. Larger Than PDI*
Boyd (1930)	Lorraine Co., Ohio		White	2.7	0
P. F. Russell (1935)	Massachusetts	613	White	3.9	
	Philippines	1243	Native	2.8	0
Maxcy, Barber and Komp (1927)	Washington, D. C.	193	White	15.0	1.5
	Hagerstown, Md.	245	White	11.6	.5

Interpretation of Spleens Palpable on Deep Inspiration

The significance of spleens palpable only on deep inspiration is debated, particularly when these are associated with low blood slide indices. In Cuba, Carr and Hill (1942) found a spleen rate of 11 per cent in 71,376 white school children as compared with a thick blood film rate of only 0.4 per cent in 34,478 of these children. In 19,391 negro children the spleen rate was 5 per cent whereas the thick blood film rate was 0.8 per cent in 7,902 of the same children. Of the spleens palpated 92 per cent were felt only on deep inspiration. These authors consider their results to conform with the opinion held by Maxcy (1927) and others, that only spleens palpable on normal respiration represent malaria and that a spleen rate less than 8 or 9 is within the range of normal.

On the other hand there is evidence that palpable spleens including those palpable only on deep inspiration do represent malaria when present in more than 5 per cent of active children. This is the experience of Russell (1935) who lists 6 points in favor of this tenet:

(1) The blood parasite rate is higher in the "PDI" group than in the negative group.
(2) Quinine affects the size of spleens palpable on deep inspiration.
(3) Surveys in non-malarious areas have less than 5 per cent spleens that are palpable.
(4) 514 cases with palpable spleens after taking an uncontrolled

amount of treatment showed return of spleen size to "PDI" or negative.

(5) The more malarious an area is, the more "PDI" spleens there are.

(6) No area has been found with greater than 5 per cent spleen index where evidence of previous transmission of malaria or of the entrance of infected persons into the community was not at hand. (Thus, where Carr and Hill (1942) found an 11 per cent white spleen index for the years 1935-41, there was an average of 2 cases of malaria reported each year per 1000 population. Here the reported morbidity rates had dropped in the last three years.)

In hypoendemic regions, small spleens constitute a large proportion of the total number with enlargement. As Ross (1910) expresses it, the average enlargement of the spleen is a reflection of the rate of transmission; the "average enlarged spleen" is an index of the average amount of illness caused by malaria in a community. If, due to a low rate of transmission the "average enlarged spleen" is small, the desirable epidemiological method would appear to be in detecting slight enlargement. Darling (1924) was one of the first to urge the use of the most sensitive method possible in malaria splenometry. This author noticed that in his 1924 survey (Darling, 1926) the "PDI" category increased at the expense of larger spleens although the malaria prevalence diminished that year.

Relationship of Spleen Index to Blood Index

Carley and Balfour (1929) and others expressing doubt as to the significance of "PDI" spleens were influenced in part by the fact that thick blood film surveys in their experience agreed better with spleen indices when the latter included only those spleens palpable during normal breathing. Thick blood film surveys, however, err in that they do not reveal all of the infections, and possibly the higher rates indicated by the sensitive method of spleen surveys more accurately approach the true prevalence. An estimate of the inaccuracy of parasite indices under hyperendemic conditions is provided by Barber (Williams, 1936; Carley and Balfour, 1929), who judged on the basis of repeat examinations in a group of known positives, that in not more than half the carriers was the thick blood film positive at any one time. The majority of malaria cases in Southern United States are thought to be of the chronic type (Forde and Forde, 1939; Zeigler et al, 1923) and in these, the so-called "haemic relapse" occurs infrequently. Determining the blood index in a group having positive histories, Rice and Watson (1943) estimated that with a 1 per cent thick film index there was a 9 per cent actual parasite prevalence and with a 5 per cent blood index a 30 per cent parasite rate. The inaccuracy of blood slide surveys then is exaggerated by low malaria prevalence. The blood slide rate is higher the larger the spleen size judging from the reports sum-

marized in Table 4. P.D.I. groups are seen in Table 4 to have higher blood rates than do spleen negative groups.

TABLE 4

Parasite rates according to Spleen size.

Author	*No. with Neg. Spleen*	*Per Cent Blood Pos.*	*No. PDI Spleens*	*Per Cent Blood Pos.*	*No. with #1 Spleen*	*Per Cent blood Pos.*	*No. with larger than #1 Spleen*	*Per Cent Blood Pos.*
Carr and Hill (1942)	33,783	0.2	7,904	1.0	576	7.8		23.3
Maxcy, et al (1927)	601	3.8	10	6.2	28	14.3	4	25
Darling (1924)	338	11.2	51	35.3	126	34.1	82	Greater than 50
Darling (1925)	267	11.2	17	23.5	104	28.8	58	Greater than 50
Russell (1934)	521	1.7	114	54.4	161	64.8	34	74.7
Boyd (hyperendemic area) (1926)	884	24.9			2046	26.9	970	34.8
(hypoendemic area)	5,703	14.7			272	27.2	69	13.0

Table 5 shows spleen and blood rates that are fairly comparable. Here all P.D.I. spleens were excluded because the children were examined (mostly) while standing and during normal respiration.

Table 6 porvides, for comparison, results of spleen and blood surveys when the more sensitive method of splenometry was used. The spleen rate is considerably higher in comparison to the blood slide rate with the latter method.

Barber (1936) offers an explanation of the frequently occurring discrepancy between spleen and parasite indices. He says "The parasite index gives us merely the proportion of the population in which parasites are numerous enough to be detected in a small sample of blood . . . It is possible that a degree of parasitism sufficient to maintain or increase the spleen index exists in many individuals apparently negative, especially in a region of high transmission." When the rate of transmission diminished in Greece after 1932, Barber observed that the blood parasite index fell more than the spleen index, the latter remaining nearly constant in 5 to 15 year old children, though dropping in the one to five year old group. In explanation he states, "We have also to reckon with the possibility that an enlarged spleen may persist for some time after parasites have disappeared, its slow decrease being due to its fibrous and other pathological conditions . . . older children with a longer

TABLE 5

Blood and Spleen Rates in School Children in the U. S. Excluding Sp leens Palpable on Deep Inspiration.

Author	*Date*	*Place of Survey*	*No. Spleens Exam.*	*Per Cent Pos.*	*Bloods exam. in group with Spleen Exam.*	*Per Cent* Positive
Derivaux (1917)	Apr.-May	Lake Valley, Ark.	141	2.8	141	17.0
Barber and Coogle (1921)	Feb. 1920	Mitchell Co., Ga.	1261	2.1		
" (unpub. data)		" "	80	5.0	70	5.6
"	Oct. 1920	" "	1181	1.3	318	4.7
" October, 1920		Counties adjacent Mitchell Co., Ga.	600	4.5		
Maxcy and Coogle (1923)	Dec. 1922	South East, Mo.	929	9.4	362	2.5
	Nov. 1922		447	4.3	183	3.8
	Jan. 1923		890	7.9	395	7.3
	Feb. 1923		64	4.7	57	0.0
		Totals	2330	7.7	997	4.5
" "	Feb.	Highlands, Mo.	84	0	21	0
" "	March	Kentucky	363	4.4	176	0
" "	May	Houston Co., Ala.	235	4.3	98	2.1
		Escambia Co., Ala.	532	1.3	390	1.0
Veldee (1923)	March, 1923	S. E. Mo., Dunklin	880	5.1	880	3.0
Maxcy, et al (1927)	1926	LeFlore Co., Miss.	930	3.74	795	3.0
Carley and Balfour (1929)	1927-1928	Humphries and Sunflower Co., Miss.	838(W) 1168(C)	4.0 4.1	1004* 4487*	1.0* 1.7
Coogle (1927)	1923-1925	Ark., Ga., S. C., Miss., La.	6500	6.0	6500	6.0

*Includes spleen group and others

TABLE 6

Blood Rates in the U. S. compared with Spleen Rates, including Spleens Palpable on Deep Inspiration

Author	*Date*	*Place*	*No. Spleens Exam.*	*Per Cent Pos.*	*No. Bloods Exam. in Group with Spleen Exam.***	*Per Cent Pos.*	*Per Cent of Palpable Spleens PDI*
Maxcy et al (1927)	1926	LeFlore Co., Miss.	930	22	795	3.0	8.3
Darling (1925)	1923	Lee Co., Ga.	571	40.8	571	25.2	20.3
	July-Sept. '24	Leesburg, Ga.	483	37.4	483	22.5	
	Oct.-Nov. 1924	Leesburg, Ga.	339	69	339	52.5	
Hanson, Boyd and Griffitts (1935)	1931	Florida (White)	3009	34.2			
	1932	(Negro)	2502	15.2			
	1934	Florida			15,257	6.1	
John E. Elmendorf (Personal Communication)	1942	Florida	6497	25.6	1,200	.08	
Spicknell and Carrozzo (unpublished data)	1942	Beaufort, S. C.	40	25	40	0.0	80
		Tennessee (White	184	17.9	184	0.0	71
		White	185	11.5	185	0.0	94
		Negro)	48	6.3	48	0.0	100

*Plantation survey of negro children in hyper-endemic area; others are school surveys.
**All reports but Darling's are on thick blood film examinations.

or more severe exposure of infection retain splenomegaly longer than do younger ones. At all events, with a diminution of transmission the spleen index in a population eventually falls." A similar conclusion is drawn by Balfour (1935) from extensive experience in Greece. He writes, " . . . one arrives at the conclusion that the spleen findings usually present an average picture of conditions which existed during the previous two or three years . . . the blood index of a group is more susceptible to change and more nearly reflects the conditions which existed in months immediately preceding the examination."

Earlier, Darling (1924) had said that there are many positive spleens without positive blood films, because the splenomegaly lasts some time after an attack. Parenthetically, it is to be noted that a lag in spleen rates may account in part for the relatively low spleen indices compared with parasite indices that are reported in epidemic malaria (Butts, 1937; Feng, 1936). The ratio of spleen to parasite rate may then be a function of the phase of the epidemiologic cycle.

Relation of Spleen Index to Medication

The United States differs from other countries in having the highest per capita annual consumption of anti-malarials. The effect of quinine on the spleen indices, either in complete dosage, or in irregular inadequate dosage, may differ sufficiently from its effect on the parasite index to account in part for a disagreement in these two sets of data. Evidence on the subject is difficult to evaluate, since field conditions can scarcely be controlled in respect to all factors except that of treatment. Some light was thrown on the matter by the investigation of Collins (1926), in which 74 Georgia children were given standard quinine therapy during April and May, 1924 and 1925. The treated cases were compared with 74 untreated control cases, in respect to blood parasite densities and spleen mass. Full clinical treatment in a year when reinfection was minimal had the effect of accelerating the decrease in spleen mass; one-half the standard treatment reduced the spleen size less than did the standard course. After the full clinical treatment was concluded, the average spleen mass further decreased and then remained at the "palpable on inspiration" level. Following the substandard treatment spleen mass remained constant. In the same year, under adequate treatment, parasite indices fell to zero and remained low; under inadequate treatment parasites did not disappear, but fell to a low level and remained there. In this report as in others, spleens of large size responded to treatment more than did smaller ones.

Veldee (1923) gave quinine to a few cases and stated that

irregular dosage rendered the blood sterile, whereas complete treatment removed the positive spleen (palpable by crude method). It is of more theoretical than practical interest that quinine injected into the jugular vein of a dog causes contraction of the spleen (Roth, 1912).

Darling (1925) gave treatment to children in a negro family and observed that the spleen receded to "PDI" in one child whose slide was negative, but remained unaltered in the sibling whose slides also became negative. Ten weeks of standard treatment of 50 negro children, he also observed, caused general diminution of the size of the spleen, a trend which reversed on discontinuance of tratment. Reinfection was probable.

Maxcy and Coogle (1923), believe the spleen rate to be a better measure of malaria prevalence than the blood film rates, in areas where inadequate self-medication with chill tonic prevails, but not where adequate quinine dosage is customary. The variation in spleen rates which they observed in several comparable endemic areas of Southern United States were thought to have been caused in part by variation in habits of taking quinine. Because of the large amount of treatment taken, Williams (1936) considers the spleen survey an unsure method of finding infections in the United States.

One is tempted to consider whether irregular quinine administration and a declining rate of transmission do not act similarly in favoring a high spleen-parasite ratio. The difference between a positive and negative blood slide is more susceptible to these influences than is the difference between a palpable or non-palpable spleen. Under such conditions the tendency is not to eliminate parasites from the body but rather to keep the parasite density at a level where the splenic defense reaction is constantly stimulated without being overtaxed. This would be so where medication had removed parasites from the blood but not from fixed tissue cells particularly in the spleen.

Duration of Splenic Enlargement after Infection

Of some importance is the matter of how long splenomegaly relative to parasitism persists. The acutely enlarged organ recedes quickly under treatment (Veldee, 1923). The enlargement generally disappears within nine weeks after spontaneous termination of a therapeutic course of malaria, the duration and degree of splenomegaly being proportional to the duration of the clinical attack, (Stratman-Thomas, 1935). Factors such as fatigue, malnourishment, improper medication, and exposure, however, hinder the body's defense reaction in unhospitalized cases and may be expected to prolong the splenomegaly. A report from Gill (1914) bears

on this point. A Punjab village affected by the epidemic of 1908—but not by a subsequent epidemic—had a spleen index of 75 per cent the first year after the epidemic and of 59 per cent the second year; 21 per cent the fourth year; 10 per cent the fifth year; and 1.4 per cent the sixth year. Relapses (and probably also reinfection) have been shown to cause a chronic enlargement with fibrous growth which recedes slowly (Stratman-Thomas, 1935; Boyd 1941). Hence the assertion by Balfour already referred to, that spleen indices record the average picture of malaria during the previous two or three years.

It makes some difference in the demonstration of the need for malaria control, or in the interpretation of the result produced thereby, whether the survey method in use shows actually infectious cases, latent and potentially infectious cases, or healed scars of former disease. Barber (1936) and Maxcy and Coogle (1923) consider the possibility that the pathology is so permanent as to persist after eradication of the parasite; whereas Boyd (1941) and Stratman-Thomas (1941), studying hospitalized cases, look upon splenic enlargement as evidence of actual infection and hence threatened relapse. Experimental evidence of interest in this regard is that of Knowles (1934), who precipitated attacks of malaria in monkeys, long clinically healthy, by splenectomy.

Proportion of cases having Palpable Spleen—Factors Involved

Not all persons infected with malaria have palpable spleen and the percentage of palpable spleens is influenced by several factors. According to Stratman-Thomas (1935), among paretics undergoing a therapeutic course of *P. vivax* infection, there was no palpable enlargement on deep inspiration in 10, transitory enlargement lasting less than 9 weeks in 37, and enlargement to the umbilicus lasting more than 9 weeks in 4. The per cent of positive spleens found in known blood positive groups under field conditions is given in Table 7 which shows that a large proportion of cases fail to show splenomegaly.

In the Mississippi Valley, Maxcy and Coogle (1923) found 58 positive blood slides in 997 children examined, and of the children showing positive slides 50 per cent had a number one or larger palpable spleen. Feng (1936) reports 50 per cent of 292 positive blood slides were spleen positive in an epidemic in Kwangsi. Of 146 blood positive cases in a group examined by Hackett, 81.5 per cent had palpable spleens (Hackett, 1944).

By comparing post-mortem spleen weights with ante-mortem palpation, Oudendal (1925) and Clark (1928) arrived at the figures listed in Table 8 which give some idea of the proportion of enlarged spleens likely to be missed in adults. The average 20 year old male white person has a spleen weighing about 170 gms.

TABLE 7
Percentage with Spleen Enlargement in known Parasite Group

Author	*No. Blood Pos. with Vivax*	*Per Cent of these Spleens Pos.*	*No. Bloods with Falciparum*	*Per Cent of these Spleens Pos.*	*No. Bloods with Malariae*	*Per Cent of these Spleens Pos.*	*Comment*
Barber, Mandrekas and Rice (1937)	60	47.3	120	75.2	2	100	Survey in Greece
Barber and Rice (1937)	91	33.2	165	76.7	4	80	Egypt
Wui and Poty (1935)	72	45	126	62.3	6	50	China
Barber, et al (1936)	371	68.5	496	98.5	393	85.5	Greece China
Hackett (1944)	21	52.4	33	94.0	11	91	Albania
Boyd (1926) hyper-endemic area 843	843	80.4	205	78.9			Brazil
Hypo-endemic area	778	9.4	144	6.9			

(100-250 gms.) (Gray, 1942). The average spleen in the negro weighs less and in the Japanese is said by Oudental to approximate 83 gms. Oudendal lists several factors as determining the palpability of the enlarged spleen:

(1) Distance from diaphram to the left coastal margin.
(2) Length and width of spleen, its thickness and consistency and the direction of its long axis.
(3) Thickness and tension of abdominal wall.
(4) Examiners deftness.
(5) Abnormal filling of abdomen from other causes.

TABLE 8
Results of Spleen Palpation in adults according to Spleen Weight.

Author	*Race*	*Spleen weight post-mortem*	*Number examined*	*Per Cent Palpated ante-mortem*
Oudendal (1925)	Japanese and Chinese	110-250 gms.	165	11.45
		250-400	103	37.7
		400-870	87	58.7
		870-2147	28	96.4
Clark (1928)	Negro	100 or less	14	7.1
		101-200	53	30.2
		201-300	53	60.4
		301-400	30	86.7
		401-500	22	86.4
		501-600	8	87.5
		601-1100	8	100

In Table 7 is also to be noted that *P. falciprarum* infections, when this is the predominant parasite, have a higher spleen rate than do *P. vivax*, an observation in accord with the opinion of Bar-

ber (1937). Barber believes that repeated infections of *P. falciparum* and *P. malariae* tend to increase the size of the spleen continuously, whereas this is not a feature with *P. vivax* (Barber et al, 1936). This is important in the United States where *P. vivax* predominates. Kitchen (1941) states that the splenomegaly in primary therapeutic *falciparum* infections does not reach the proportion it does in non-immunes with vivax infections—due, he believes, to the shorter duration of primary *falciparum* cases. Stratman-Thomas (1935) has noted the degree of enlargement in primary *vivax* patients to be proportional to the duration of the attack.

Some difference in spleen response has been observed according to age of the patient. Stratman-Thomas (1941) says, "In infants and young children, *P. vivax* produces a greater degree of splenomegaly more rapidly than in adults." A higher rate of spleen enlargement in the two to five year old group and a lower rate in the ten to fifteen age group suggests high endemicity. Conversely a lower rate in the younger class coincides with low prevalence of malaria (PHR 1921; Hackett 1944). Enlargement of the organ can be most easily detected in the age group between 2 and 12, because of better abdominal relaxation (Darling 1924) and less well-developed musculature. For this reason and because they are more accessible, the usual practice is to conduct spleen surveys on school children.

The spleen reaction differs in the white and negro races. Negroes are said to have more of an immunity to clinical manifestations than to parasitism and accordingly to have low spleen rates in proportion to the degree of parasite prevalence (Hackett, 1941). Results obtained by Clark (1928) on post mortem spleen weights of malaria cases showed that West Indian negroes had less enlargement than Latin Americans. The smaller size of the normal negro spleen is also a factor (Gray, 1942). The experience in this country has been that the negro race gives a lower index than does the white, a fact which is demonstrated by limited data in Tables 5 and 6, and be the findings of Maxcy (1927). Andrews (1941 recommends doing spleen surveys on white, and blood slide surveys on colored children.

According to Boyd (1941) the rate of increase in size of the spleen is more rapid in those with some immunity. A very large spleen may be indicative of resistance because the incidence of fever in cases with #4 spleens was found to be less than in those with #3 spleens (Boyd, 1926).

Attention should be directed to the role nutrition plays in determing the reaction of the spleen to malaria. The thought that malnourishment exaggerates the reaction has been frequently expressed (Darling 1924), but without experimental confirmation.

Several nutritional factors are essential to the functioning of the hematopoietic system, deficiency in one or more of which may affect the response of the spleen to paraistism. However, it should be remembered that famine also increases the amount of malaria.

Discussion

In practical application, splenometry seems to have been neglected recently in the United States, partly because the significance of slight splenic enlargement is not agreed upon, perhaps also because the technique involved in measuring spleens palpable on deep inspiration is not generally familiar to public health workers. Moreover, malaria control projects in the United States, where the vector is known, can be administered according to information on *Anopheles quadrimaculatus* breeding according to recent history of malaria in the community. The advocate of spleen surveys would suggest that his is the best method for determining objectively the recent history of parasitism in the community. Fort says (Boyd 1926) " . . . if we examine the spleens of small children in a country school, in a few minutes we know more about malaria in that section than microscopic examinations and many doctors can tell us. If we separate the children with enlarged spleens, map where they live, and drive to their homes during the warm season, we find *quadrimaculatus* in the house. Larvae can be found in the ponds about — thus in a few hours we can trace infection to its source and give instructions about drainage, oiling, etc." Splenometry offers an objective, rapid, inexpensive and relatively sensitive method of determining the need for malaria control. However, it is less specific than the blood slide method, does not reveal the parasite species and therefore needs to be checked frequently by simultaneous blood slides. Reflecting as it does the average picture for the previous two or three years, then spleen index would not be sensitive to moderate changes of the preceding season. It may not provide the most satisfactory check on the effect of recent control measures, although a shift in the proportion of small or large spleens and in the rates in the 2-5 year old group compared with the 5-15 year group may reflect recent changes in the rate of transmission.

Greatly needed is a means of anticipating events in malaria. Experience has shown that rates follow cycles of a number of years, that during the down slope of the cycle epidemic flare-ups are infrequent and readily suppressed, but that on the upswing localized outbreaks tend to coalesce into a generalized rise. What the factors are behind such fluctuations has received considerable attention. Mosquito density may not be the sole important factor. An opportunity to observe *quadrimaculatus* "anophelism without malaria" presents itself in the widespread entomological surveys being conducted in war areas. The impression gained is that *quadrimaculatus*

density in the South does not correspond to the all-time low mark of malaria prevalence; however, quantitative comparisons with former years cannot be made. Faust (1942) believes that economic cycles, by influencing the nutrition, medication, and shelter of the population and the amount of malaria control work accomplished, very largely determine malaria cycles. Gill (1938) considers it possible that plasmodia spontaneously, or as a result of unknown forces, perhaps meteorological, pass through cycles of increased, then decreased, perfection of performance. Other authorities place primary importance on the matter of human resistance to the parasite. The "premunition" to malaria lasts up to five years and the suggestion has been made that as it declines in a population group, rates commence to rise. Spleen enlargement appears to be related to immunity in an ill-defined way. Possibly the spleen index in the population, particularly in its relationship to the blood slide index, could provide information as to when the bottom of the cycle has been reached.

Summary and Conclusions

1. The spleen survey of school children may provide a useful tool in ascertaining the malaria prevalence under hypoendemic conditions if its limitations as well as advantages are considered. (a). A recent epidemic of one of the childhood diseases and possibly a high rate of upper respiratory tract illness can appreciably increase the spleen index. A proportion of presumably healthy children have spleens palpable on deep inspiration. Although available reports on the matter are not in agreement and more information is needed, it can be said that spleen rates as high as 15 per cent may occur in areas where there is no malaria. These will be almost exclusively spleens palpable only on inspiration. (b) Spleens palpable on deep inspiration are caused by malaria and may be the only measure of prevalence where rates are low or have been progressively decreasing. These spleens can be included to advantage in the spleen survey by considering rates higher than 15 per cent—even if all are P.D.I.'s—as significant of malaria.

2. The ratio of spleen index to thick blood film index varies according to the technique of spleen examination and according to the malaria transmission rate. Reports from this country, in which P.D.I. spleens are included, give higher spleen than blood rates — in some cases more than 15 per cent higher. The experience of several authors has been cited in which a fall in the malaria rates over several years resulted in greater decline in blood than spleen indices — a situation comparable to that recently seen in the United States.

3. Quinine medication causes a decrease in spleen mass;

but possibly it tends more to produce a negative blood slide than to render the spleen non-palpable. Full clinical treatment has been found to decrease spleen mass more than sub-standard dosage. Spleens of large size have been observed to respond to quinine more than smaller ones.

4. The duration of splenomegaly in malaria cases is determined by a composite of several influences. Enlargement subsides 2-3 months after a therapeutic course of malaria but is prolonged by relapse. The spleen index in a community has been observed to remain elevated several years following a malaria epidemic. The spleen index thus can reflect the rate of transmission during the preceding several years.

5. The percent of cases having palpable spleens depends among other things upon race, age, and parasite species. Greater enlargement occurs in the white than in the colored race. The splenic reaction to malaria is thought to be more marked in children than in adults. Therapeutic infections with *P. vivax* appear to cause the greater degree of enlargement. In the field *P. falciparum* has the higher spleen rate when it is the predominant type.

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ON THE PROBABILITY OF SOLDIERS WITH PACIFIC *PLASMODIUM VIVAX* MALARIA INFECTING *ANOPHELES QUADRIMACULATUS*

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In another paper* it was shown that some *Plasmodium vivax* strains from Pacific war areas could be transmitted by *Anopheles quadrimaculatus*. Since these studies were carried out under approximately optimum conditions for mosquito infection, it seemed desirable to determine the probability of transmission of such strains. This was done through the cooperation of the staff of Kennedy General Hospital, where a large number of patients were available on a special malaria research service. The results of this study are the basis of this report.

Materials and Methods

Lots of approximately 25 newly emerged *A. quadrimaculatus* were applied to patients with *P. vivax* infections presumably acquired in Pacific war areas. All were clinically ill with these infections, or had an asymptomatic parasitemia when the mosquitoes were fed. The mosquitoes were applied without reference to gametocyte levels, but a record of this and other variables likely to influence the infection of the mosquitoes was made. Blood films made at the time each lot of mosquitoes was fed established the presence of parasitemia. Parasite counts were made by the technical staff of Kennedy General Hospital.

Patients who had received one or two 0.6 gram doses of either quinine or totaquine were included, the mosquitoes being applied about one hour after treatment. When two doses had been received, the interval between doses was 6 hours.

Subsequent to feeding, the mosquito lots were incubated at 75° to 82° F. with a relative humidity of approximately 85 per cent. The salivary glands of all mosquitoes which died after an incubation period of 11 days were examined for sporozoites. At the end of 15 days all mosquitoes remaining in a lot were examined for salivary gland infections.

Results

The term "relapse" is used in this paper to describe the reappearance of parasitemia, with or without clinical expression, subsequent to the first known appearance of parasitemia. In all cases

*Robert Briggs Watson. Observations on the transmissibility of strains of *Plasmodium vivax* from Pacific war areas by *Anopheles quadrimaculatus*. Am. J. Trop. Med., in press.

included in the study there had been clinical expression of this initial parasitemia, the attack occurring usually within 30 days after discontinuation of suppressive therapy with atabrine.

The relapse rates of infected individuals is the basic factor determining their infectiousness to mosquitoes; consequently, the relapse rates of the patients used in this study are of epidemiological importance. These rates were computed from the accumulation of the number of months and the number of relapses since the initial attack. It will be noted from table 1 that most of the patients acquired their infections in the "A" Islands. The relapse rate of 0.73 per month for this group is the highest, and the most stable statistically, of the three listed. It indicates that these men experienced an average of one relapse about every 6 weeks since their initial attacks. While the relapse rates for infections acquired in the "C" Islands and in "B" are not statistically stable, they are not unlike the "A" Islands rate.

TABLE 1
The relapse rate of patients by origin of strains

Origin	Cases	Number		Relapse Per Month
		Case Months	Relapse	
A	76	971	705	0.73
B	7	115	55	0.48
C	4	63	41	0.65
All Sources	87	1149	801	0.70

It would appear from table 2 that patients with "B" infections are more likely to infect mosquitoes than patients with "A" Islands or "C" Islands infections. However, the difference between the gland infection rates for "B" and "A" Island infections is not statistically significant*.

TABLE 2
The occurrence of salivary gland infections in relation to the origin of the *Plasmodium* strain

Origin	Cases	Gland infections		Infection rate	
		+	−	Per cent	St. Er.
A	104	147	1124	11.57	0.897
B	9	21	88	19.27	3.778
C	4	0	46	0.00	———
Total	117	168	1258	11.78	0.854

The data in table 3 indicate that during the time period when the patients were experiencing their sixth through their fifteenth relapses they were most infectious to mosquitoes. On the basis of relapse frequency, this means that the patients were most infectious

*The criterion of significance used in this paper is 2.5 sigmas.

for the period beginning about 8 months after their primary attacks, and for approximately the next 13 months thereafter. Patients with less than 6 or more than 15 relapses were much less infectious to mosquitoes. Doubtless the infectiousness of individuals in these various categories is correlated closely with gametocyte levels, but a numerical expression of such a correlation is not possible because of the lack of sufficiently stable data on gametocytemia.

TABLE 3
The influence of the number of relapses on the infectiousness of patients to mosquitoes

Number of relapses	Cases	Gland infections		Infection rate	
		+	—	Per cent	St. Er.
1-5	11	7	122	5.43	2.00
6-10	71	120	751	13.78	1.17
11-15	26	36	282	11.32	1.78
16 and over	9	5	103	4.63	2.02
	117	168	1258	11.78	0.85

The data in table 4 seem to show that individuals with asymptomatic parasitemia were more likely to infect mosquitoes than those who were clinically ill. However, the difference between the infection rate of mosquitoes fed on such patients (17.7 per cent) and that for those clinically ill and receiving no treatment (9.9 per cent), the greatest difference observed, may have occurred fortuitously (1.9 sigmas). Also, it seems evident that two 0.6 gram doses, 6 hours apart, of either quinine or totaquinine do not effect gametocytes sufficiently to prevent mosquito infection.

TABLE 4
The occurrence of salivary gland infections in relation to clinical activity and treatment

	Clinical State	Cases	Gland infection		Infection rate	
			+	—	Per cent	St. Er.
	Asymtomatic Parasitemia	7	17	79	17.7	3.89
	No treatment	33	42	382	9.9	1.45
III	One dose	39	54	419	11.4	1.46
	Two doses	38	55	378	12.7	1.60
		117	168	1258	11.8	0.85

From the data in table 5 it will be noted that the chances of a mosquito becoming infected tends to vary directly with the number of gametocytes in the infecting blood meal. This is, of course, according to expectations. However, of the mosquitoes which took blood meals at times when no gametocytes were demonstrated microscopically in the peripheral blood, 7.4 per cent developed sporozoites. This circumstance is probably due in part to inefficiency of technicians; nevertheless, it may indicate also that gametocyte levels low enough to escape detection on routine examination of blood films

are still sufficient to produce salivary gland infections.

Discussion

Everyone who has had an opportunity to study *vivax* infections from the south and southwest Pacific war areas, with whom the writer has talked, is agreed that they relapse more frequently than in-

TABLE 5
The relationship between gametocytemia at the time of blood meals and the development of salivary gland infections

Quality of salivary gland infections*	Gametocytes/cmm. blood											
	0		1-100		101-200		200+		Undet.		Total	
	No	%	No	%	No	%	No	%	No	%	No	%
—	712	92.6	153	88.4	178	86.4	155	76.7	60	78.9	1258	88.2
+	38	4.9	19	11.0	19	9.2	23	11.4	9	11.8	108	7.6
++	12	1.6	1	0.6	6	2.9	10	5.0	3	3.9	32	2.2
+++	5	0.7	0	0.0	2	1.0	4	2.0	2	2.7	13	0.9
++++	2	0.3	0	0.0	1	0.5	10	5.0	2	2.7	15	1.1
All positives	57	8.4	20	11.6	28	13.6	47	23.3	16	21.2	168	11.8
Total	769	100.0	173	100.0	206	100.0	202	100.0	76	100.0	1426	100.0

* — Negative
\+ Very few sporozites
++ Light infection
+++ Moderately heavy infection
++++ Very heavy infection

fections with *vivax* strains indigenous to this country. This circumstance seems to warrant the speculation that ability to relapse frequently is a biological characteristic of the strain, or of some strains, of *P. vivax* producing the infections. Otherwise, it must be supposed that sporozoite dosage, atabrine suppression, or some other variable common to these infections is responsible for relapse frequency.

The general experience in Army hospitals in this country is that, with any given relapse, a patient has about six chances out of 10 of experiencing another relapse. Therefore, it can be seen that a relapse rate curve for any group of infected soldiers is asymptotic. While some patients apparently are "cured" after a few relapses, others may have 20 or more at intervals of 4 to 8 weeks.

Under the condition of this study it appears that the over-all chances are about 7 to 1 against the development of a salivary gland infection by an *A. quadrimaculatus* specimen which takes blood from an individual with parasitemia. By and large, the geographic origin of the strain of *vivax*, the clinical state of the patient and the administration of two 0.6 gram doses of quinine or totaquine do not modify this generalization appreciably. It is believed that this estimate is conservative. The study was done from 1 January to 30 June 1944. Mosquitoes had to be transported about 10 miles from the hospital wards to the laboratory. While they were protected as much

as possible from atmospheric temperatures, some lots experienced brief exposure to temperatures of less than 40° F. after feeding. These lots did not develop gland infections, possibly as a result of exposure to cold. Since this variable could not be appraised accurately, no account has been taken of it.

Individuals experiencing their 6th to their 15th relapses were found to be about twice as likely to infect mosquitoes as individuals with earlier or later relapses. This finding may be of considerable epidemiological significance for the following reasons. This period of greatest infectiousness, in terms of elapsed time, would be from 8 to 21 months after the acute initial attack. Assuming that the initial attack was experienced shortly before or after the return of a soldier to this country, it seems reasonable to suppose he would return to civilian life on leave, or permanently, during the period of his maximum infectiousness to *A. quadrimaculatus*. If return to civilian life means also that the soldier becomes accessible to the biting of *A. quadrimaculatus*, then his presence in a community may result in the transmission of his infection to other residents of the community.

Summary

Anopheles quadrimaculatus mosquitoes were fed on soldiers with relapsing *P. vivax* infections presumably acquired in Pacific war areas. Feeding of mosquitoes was done without reference to gametocyte levels, number of relapses experienced, origin of strains, or any other variable. Some patients had received two 0.6 gram doses of quinine or totaquine before mosquitoes were fed. After incubation for 15 days in an air-conditioned room these mosquitoes were examined for salivary gland infections.

An over-all infection rate of 11.8 per cent was found for 1,426 mosquitoes which had been fed on a total of 117 cases, representing 87 different patients. The highest infection rate (17.7 per cent) was obtained in lots of mosquitoes fed on patients with asymptomatic parasitemia. The infection rates for mosquitoes fed on patients who had received treatment were not significantly different from those fed on patients who had received no treatment.

It is concluded that many, if not all, strains of *P. vivax* from Pacific war areas can be transmitted by *A. quadrimaculatus* and that returning soldiers with such infections may be responsible for the establishment in this country of epidemic or endemic foci of imported *vivax* strains.

Acknowledgment

This study was made possible by the courtesy and cooperation of the staff and the patients of Kennedy General Hospital. It is a

pleasure for the author to acknowledge his indebtedness and to express his appreciation of this help, especially to Brig. Gen. Royal A. Reynolds, Commanding General; to Lt. Col. Vernon O. Powell, Chief of Medical Services; to Maj. Newell R. Ziegler, Chief of Laboratories; and to Capt. Harry Most, in charge of malaria research wards.

A DISCUSSION ON CARIBBEAN MALARIA CONTROL*

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I. Introduction

Malaria and anti-malaria activities in the Caribbean have long been of interest to a few persons in this country with a professional interest in tropical medicine. Recently many others have become interested as well. These include most professional malaria control workers in this country, the general public health profession, and several Federal agencies. The establishment of many Army and Navy Bases in the Caribbean, the Good Neighbor Policy in Latin America, the application of Lend Lease to include the countries and possessions of our allies in the Caribbean, and the forced production of critical agricultural and forest products in Latin America have all contributed to this trend.

While the end of the war will result in sharp curtailment of many war inspired activities and a consequent decline of interest in some quarters, there is little reason to expect reversion to the former status, when ignorance of the Caribbean was general among the otherwise well informed. Among other factors, expanding economic ties with Latin America will further the continuance of more intimate commercial, cultural and socio-economic relations than prevailed before the war.

Present indications are that field workers in malaria control also will have a continuing interest in the Caribbean. Maintenance of the prevailing down-trend in malaria occurence in this country may dictate for many a choice between employment outside of the United States in their chosen field or a change of profession. Those concerned with the attack on residual malaria in this country will be guided increasingly by work in nearby areas outside of the United States where the disease occurs in readily measurable quantity. Perfection of new anti-anopheline technics and procedures requires evaluation and proof in terms of malaria reduction. Sound standards of performance and keen interest in accomplishment on malaria control operations can be sustained generally only when work is performed where malaria is current and prevalent. The large nearby reservoir of malaria in the Caribbean, coupled with sharply increased post-war travel, may markedly increase the hazard of introducing new plasmodial strains into this country, as well as

*This paper was presented at the annual meetings of the National Malaria Society in St. Louis, Missouri, 14 November 1944.

re-introduction of malaria into localities of recent endemicity. For these reasons, at least cursory discussion of malaria distribution and prevailence and the status of malaria control activities in the Caribbean region seems warranted.

Although countries bordering the Caribbean include Central America, Panama, Colombia, and Venezuela, this discussion involves only the Greater and Lesser Antilles and the Guiana coast of northern South America, since it is only with these areas that the writer has even fleeting acquaintance. However, the length of this sub-region alone exceeds the United States in breadth and has nearly comparable climatic variation. Annual precipitation varies from 10 inches in Aruba to well over 200 inches in the flat swamp lands of French Guiana. Elevations vary from sea level to 10,000 feet. On some islands strong winds blow constantly day and night eleven months out of twelve, while in the interior of others even zephyr breezes are seldom felt. Humidity varies in proportion.

As will be seen on Figure 1, the Guianas are part of coastal equatorial South America, and are composed of British Guiana, Surinam or Dutch Guiana, and French Guiana. Proceeding westward, the British Crown Colony of Trinidad, while an island, is

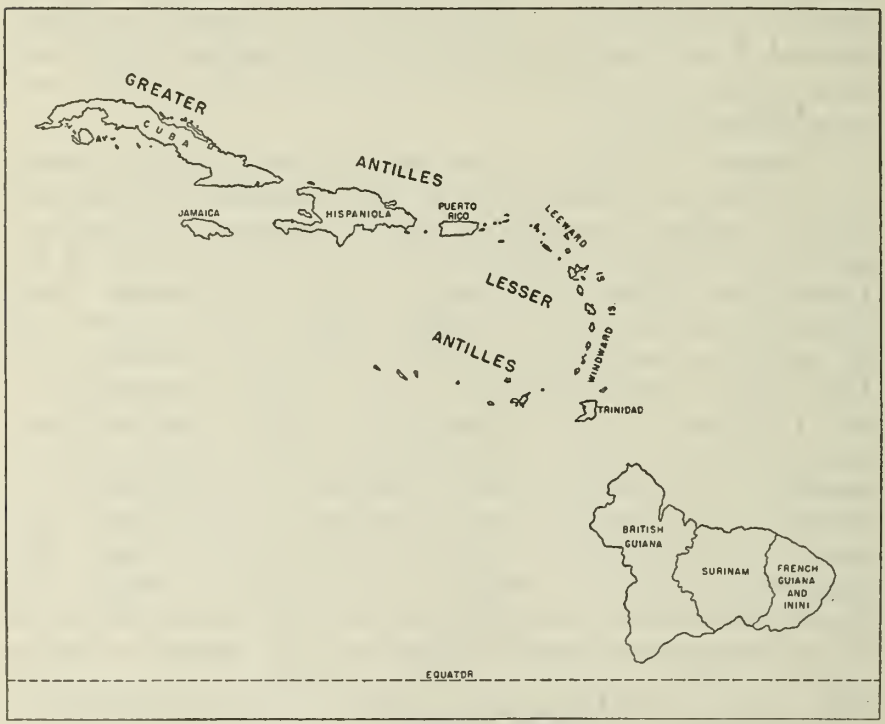

Fig. 1. The Antilles and Guianas

topographically related to the South American mainland. The Antilles Archipelago is composed of the Greater and Lesser Antilles, the latter being subdivided into the Windward and Leeward Island groups, plus scattered islands north of Venezuela. The Windward Islands include the British island of Barbados, St. Lucia and Dominica. The Leeward Island group is composed of British, Dutch, French and American possessions in haphazard assortment. Among the more important are the American and British Virgins, the British island of Antigua and St. Kitts, and the French island of Guadeloupe. The more important scattered islands of the Lesser Antilles include the Dutch islands of Bonaire, Curacao and Aruba, while the Greater Antilles comprise Puerto Rico, Hispaniola (Haiti and Dominican Republic), Cuba and Jamaica.

The total population of these areas exceeds 14 million. The composition of population by language, culture and government is shown by Figures 2 and 3.

II. Malaria Distribution and Prevalence

As might be supposed from the climatic variation, malaria is not uniformly prevalent. In some places, such as Curacao and Aruba, there appears to be neither indigenous malaria nor anopheline vectors. In others, such as Cuba, (Carr and Hill, 1942), the problem is

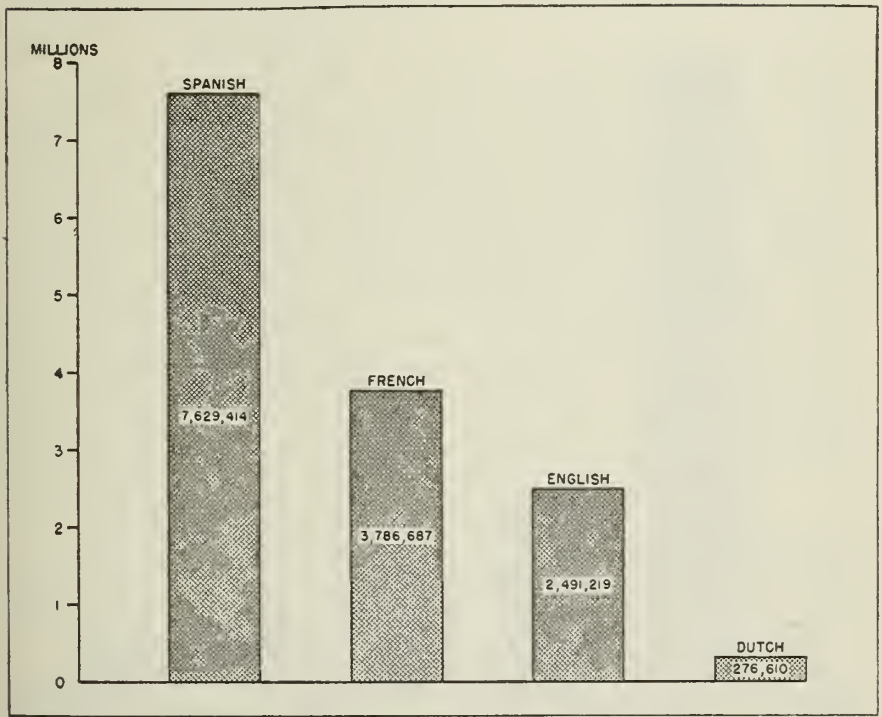

Fig. 2. Population—West Indies, Trinidad and Guianas by language and culture

one of hypoendemicity of little economic consequence, with widespread epidemics at long intervals. Other islands fill niches in the rising scale of malaria prevalence, and those at the head of the list have an endemic problem probably not greatly exceeded anywhere in the world.

The pattern of malaria prevalence is further characterized by great variation *within* as well as *between* areas. Topography is the primary determinant, but agricultural practices, precipitation, soil permeability, population density, race, and economic status, and the distribution of anopheline species are significant although frequently these too are derived from topography. The malarious islands of the Antilles, Cuba excepted, are composed of the eroded tops of mountains or mountain ranges. Relatively narrow coastal plains have been created by erosion, some valleys have been cut inland by streams, and occasional plateaus occur. Rugged slopes are commonly encountered in the interior, and these extend occasionally to the shore. Malaria is concentrated largely in the peripheral coastal plains, with inland indentations along stream terraces. Figure 4, an isorropic map of malaria mortality in Puerto Rico, agrees closely in contour spacing with a topographic map of the island, and is representative of malaria distribution in some other islands.

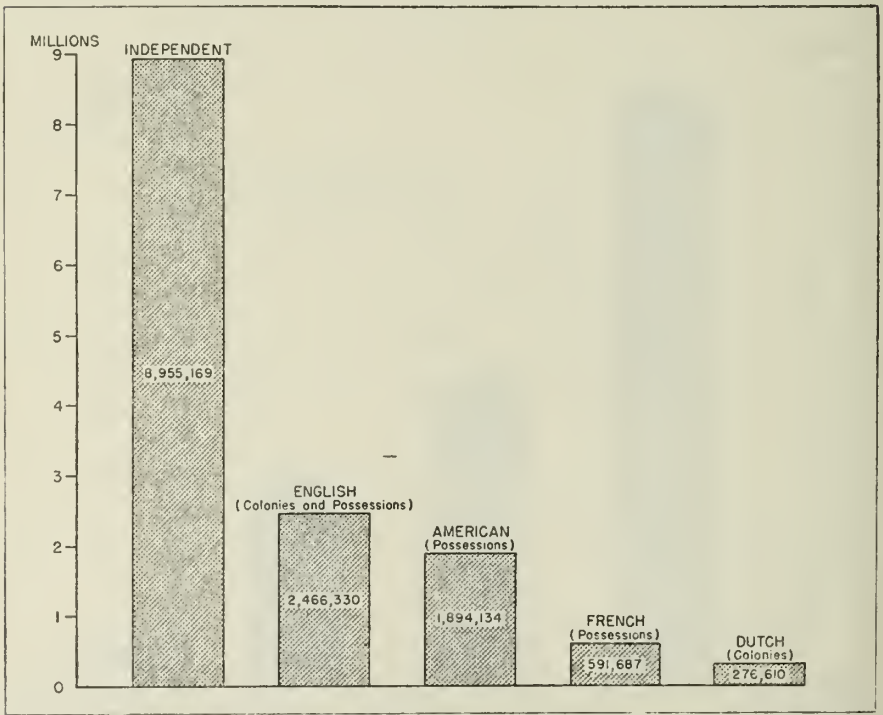

Fig. 3. Population—West Indies, Trinidad and Guianas by type of government

Thus, not only are some islands malaria free, but portions of even the most highly malarious ones are salubrious.

The Guianas by contrast are composed largely of immature coastal plain. Some elevated land is found in the interior. This is populated by nomadic Indians and a few isolated bauxite mine communities. Nearly all of the population is concentrated on or near the coast or within coastal access along the banks of navigable watercourses serving as highways through swampy jungle. Here, the distribution of malaria is determined largely by sheer presence or absence of population.

Data permitting *accurate* comparison of malaria prevalence between individual areas and with continental United States are not available. In some areas, island-wide malaria surveys have been made by health authorities, generally with the aid of the Rockefeller Foundation. Spleen and parasite indices obtained, mainly reflect the status of malaria for an individual year and may represent conditions above or below the mean malaria level. Nevertheless, they furnish a more adequate picture of malaria than is available for the remaining areas. Mortality and morbidity statistics are subject to the some deficiencies characteristic of these data in continental United States, with a few more added. In the more remote localities, medical service is rare and mortality only partially reported. But in the absence of more adequate data they possess value in assessing the malaria problem.

Through assorted data, including clinical records and personal experience, malariologists are generally agreed that in the Antilles islands, malaria prevalence is greatest in Haiti and St. Lucia. The Dominican Republic is added by some. While knowledge of malaria in two of the Guianas is limited, it is believed that all three rank with the leading islands of the Antilles. In two other islands, Puerto Rico and Trinidad, the insular-wide incidence of malaria is lower, but even here it is one of the leading causes of death, probably the commonist disabling illness, and a primary determent to economic improvement. The population of these *eight* places is seven and three-quarter millions. Since population as well as malaria is concentrated along the coasts due to lack of ports and arable land in the interior, at least five and possibly six million people live in the more intensely malarious portions.

The critical presentation of all available epidemiologic data is outside the scope of this paper, but a few of the higher rates and indices may be illustrative. In Haiti it is reported that the parasite index in one representative "high malaria" town ranged from 75 to 85 per cent in a series of surveys. According to Faust, (1941) the national 10 year average annual mortality rate was 268 per hundred thousand, based on incomplete reporting. In British Guiana,

Fig. 4. Malaria distribution—Puerto Rico, mortality per 100 000 population, based on malaria mortality at place of death 1931-39, and census of 1935

Ciglioli (1938) reported parasite indices of 54 to 78 per cent for selected localities in an extensive series. He states that one-eighth of the crude death rate on sugar estates is directly ascribed to malaria and that "the highest mortality is caused by chronic malaria—and a great many such deaths are registered under (other) diagnoses." In St. Lucia, Fox (1941) states "malaria causes more non-effectiveness than all other disease conditions in the island combined." Parasite indices in Fox's report (obtained from Earle) range from 40 to 87 per cent. Data for French Guiana and Surinam are more limited than for other places discussed. Apparently no vital statistics showing cause of death are maintained in French Guiana, except fatalities in hospitals and among felons. Scattered data give same clues as to malaria incidence however. Blood films were collected by military forces in 1943 from several hundred *able bodied adult* male laborers who were candidates for employment on emergency construction projects. Nearly all lived in Cayenne, the principal port, which French authorities advised was more free from malaria than any other place. The parasite index for this group was 32 per cent (Bolten and Henderson, 1943). The character of the terrain and the established presence of *A. darlingi* throughout the colony all contribute to the impression of high malariousness. In Puerto Rico, while the average malaria mortality rate per hundred thousand in the most recent decade was only 142, the range was from 300 to over 600 along most of the thickly populated south shore.

III. Entomology

The entire area is an entomologist's paradise. Much basic taxonomy remains unperformed due to the larger number of closely related anopheline species and complexes, and inadequacy of personnel. Many lacunae exist in the knowledge of anopheline adult behavior and larval habitat. Entomological services are even more essential to effective malaria control operations than in continental United States. Species identification of larval and adult specimens collected routinely on project operations is more difficult, particularly among the Nyssorhynchus group. Two of the four principal vectors, *A albimanus* and the *A. aquasalis - tarsimaculatus* complex, breed in a wide range of aquatic habitat, including scattered temporary accumulations and saline waters. Effective antilarval operations follow the form of a tactical campaign of suppression approaching eradication rather than a routine program of partial reduction.

IV. Malaria Control

Professional malaria control workers in this country are conditioned to viewing twentieth century malaria as a rural problem.

While incidence has been significant for many towns and small cities in malarious areas, the problem in larger cities, when present has been confined almost wholly to suburbs. State-wide urban malaria rates and even densities per square mile have been negligible in relation to rural incidence. This is in contrast to the region under discussion. Although *rural* rates are generally higher than *urban* and the number of cases greater where the rural population is large, an acute problem exists in many principal cities. These urban malaria conditions result from lack of conscious malaria control by government, the longer effective flight range of some anopheline vectors, abundance of nearby breeding places and lack of protection against adult mosquitoes.

Table 1 illustrates the relationship between urban and non-urban malaria mortality in Puerto Rico, corrected for place of residence. The term non-urban rather than rural is used to describe population living outside of urban centers of more than 2,500 population, since a mixture of suburban, small community and rural population is involved. The insular-wide urban mortality rate slightly exceeds the non-urban, and rates for cities in the population groups 10,000 - 25,000 (6 cities) and 25,000 - 100,000 (2 cities) are 218.6 and 214.8 respectively, or approximately 60 per cent greater than for all urban areas. This is because most of the larger cities are on the coast. Mortality records are also given for Ponce, the second city in size. The *urban* rate is high, but the local non-urban rate is higher. This partly answers the thought that non-urban fatalities may have been charged to the urban area through incorrect reporting of place of residence. The fact that the *density* of malaria mortality is nearly 30 times greater in the urban than in the adjoining non-urban area is significant in malaria control planning.

Public health workers in continental United States, impressed with accomplishments and procedures in the Panama Canal Zone, are apt to assume that sanitation for prevention of the predominant filth-borne and insect-borne diseases occupies a priority position in tropical public health practice. Lack of progress in disease reduction is ascribed to the magnitude of the problem and lack of financial resources.

While internal resources and health department appropriations are inadequate for effective progress in many places, they are substantial in others. In two of the eight areas on the "primary" malaria list, annual health department appropriations approximate $3.75 and $5.00 per capita. In a third, total allotments for other than indigent care last year exceeded seven millions and more than $3.50 per capita. In all eight places expenditures for physical sanitary improvement are neglible in relation to the problem and are a

TABLE 1

Puerto Rico—Malaria Mortality (1934-38) and Population

	Urban 2500 or over	Rural and Suburban	Combined
Puerto Rico			
Population (1935)	512,020	1,211,514	1,727,534
Mortality Rate	131.5	127.2	128.5
Municipality of Ponce			
Population	60,867	39,912	97,779
Mortality Rate	302.0	327.0	312.0
Average Number Deaths	183.8	120.8	304.6
Deaths per Square Mile	30.6	1.2	2.8

minor percentage of the total health budget, even where much ado is made about malaria control and other sanitation in requesting the general appropriation. The majority of the general health department funds is expended for treatment of the sick, either in institutions or clinics, and this is the only significant activity in some. Occasionally, sanitary workers are provided under various forms of local health service to inspect, recommend, consult, investigate, or even subpoena; but private resources are inadequate, tax revenues are collected and dispensed by the central government, and opportunity to promote locally financed works programs is limited.

In the island where the current annual budget of the central health department exceeds seven million dollars, the budget for malaria control and investigational activities varies from 2 to 5 per cent of the total, although malaria is one of the three outstanding public health problems. Even this modest annual appropriation however makes an imposing total over twenty years. Several millions have also been spent in the name of malaria control during the past decade by work relief agencies under health department sponsorship. But even malaria control activities, so called, are warped into conformity with the dominant policy of medical relief and the expediencies of an outstanding program of local health service. The largest item in the malaria control budget is generally for anti-malarial drugs and personal services in house to house dispensing. Funds for anti-anopheline activities are scattered widely among malarious localities with local health service. Figure 5 shows the results obtained in terms of insular-wide malaria mortality by this type of program over a thirty-two year period.

It has been stated previously that an acute malaria problem exists in many principal cities in the eight "high malaria" areas. In most of these, purposeful antilarval measures are economically feasible in terms of per capita cost. Although costly construction

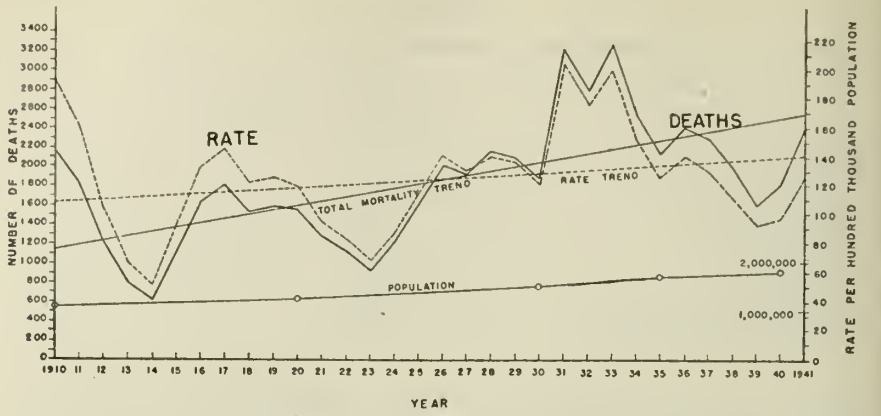

Fig. 5. Malaria mortality, Puerto Rico, 1910-1943

is involved, the greater density of malaria incidence per square mile and longevity of benefit justify the work.

By comparison, control of non-urban malaria by antilarval methods is not economically feasible, even where population density is far greater than in this country. Breeding places are generally numerous and difficult to eliminate, and the effective flight range of the vector is often longer. A greater percentage of reduction in vector population is also required for effective malaria control where malaria is highly prevalent or where a more susceptible species such as *A. darlingi* is encountered. These factors increase importantly the cost of malaria control. Exceptions occur in some highly malarious sections where vector production is limited to a few easily managed breeding places. Residual spraying to control adult mosquitoes offers promise and may extend the economic applicability of anopheline control among non-urban native populations.

Since progress in malaria control for the region as a whole resolves itself at present into an attack on urban malaria, administrative planning should be concerned with the methods and machinery needed to realize this objective. It is generally agreed that the basic method should be the permanent elimination of breeding places through drainage and related measures, for reasons of economy and effectiveness. Also, urban malaria can be controlled more positively and more rapidly by these methods than can other comparable endemic tropical disease problems.

The magnitude and character of engineering construction needed for permanent malaria control around many of these tropical cities however, places this activity in a public works catagory, comparable to, but generally less expensive than, systems of water supply, sanitary sewerage, paved streets, hospitals and other public utilities. In tropical as well as temperate areas, such work is performed generally by public works agencies or private contractors, rather than

health departments, even when the purpose is protection of the public health. These public authorities or agencies such as highway, water resources, or public works departments are established at the central governmental level, and appropriations are relatively adequate because public opinion universally supports investment of the tax dollar in permanent works, and the field of activity is clearly defined in the public mind.

In the field of public works for public health however, malaria control occupies an anomalous position. On the one hand, extensive urban malaria control construction in the tropics could not be prosecuted successfully by present public works staffs. Other types of public works construction involve static design which, once prepared and reviewed by professional specialists, can be turned over to the builder. But many tropical malaria control projects are tactical campaigns involving fluid adjustment of over-all plans throughout the period of operation. Medical, entomological and engineering services of a specialized character are essential elements of execution as well as design. On the other hand, the health department, which employs all available professional malaria control personal, is unqualified and unwilling to engage in large scale public works construction. Successful prosecution of heavy construction would require revolutionary and, from some viewpoints, obnoxious changes in health department policies, personnel and administrative machinery. Although responsibility for the performance of malaria control is associated in the public mind with the health department, tropical public health organizations are planned, administered and staffed with the objective of rendering recurrent service to the individual citizen, principally in the form of curative or preventive treatment. The medical and non-medical sanitarian play a minor role in the over-all picture. In planning expenditure of health department appropriations, the concept of allocating most, or even a large percentage of general funds for enduring physical construction at the expense of recurrent services which ultimately may be more costly, does not appear to exist in the minds of public health administrators in these areas. Increase in appropriation does not alter this situation. As a result, expenditures from public health funds for malaria control have been and will continue to be limited to meritorious investigation, small scale demonstrations, and less merited palliative activities.

Due to the diverse character of the eight countries discussed, no panacea can be offered. In the more virgin areas, continuation of the Rockefeller Foundation type of small scale pioneering demonstration under central health department aegis is indicated. In one or more, the utter lack of basic resources leaves little hope for significant activity. But in others, the large scale demonstration is justi-

fied by past pioneering and current intrinsic resources. Puerto Rico is the outstanding opportunity. There a two hundred million dollar public improvement program has been planned, and about 50 per cent of these funds are reported to be in the Treasury. Authorities and agencies have been created to meet the special needs of specific programs. Here two objectives should be sought by the sanitarian in malaria control. The first, to be performed through and with central health organization support, should be the preparation of a plan of action for sound, adequate malaria control commensurate in scale with other public improvement programs for consideration by executive and legislative branches. The second is service in the chosen instrument created or charged with execution of the task, in order that specialized medical, entomological and engineering workers may form an integrated team with the "builder" on public works construction for malaria control. It is believed that a public works authority created for malaria control is the preferred device for effective action and coalition between the scientist and the operator.

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MALARIA CONTROL IN A NONENDEMIC AREA*

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The malaria control problems which are being and will be created by the return of service personnel and the introduction of prisoners of war from all parts of the world have received a great deal of attention and discussion in the recent past. Surgeon General Parran (1944), General Simmons (1944), Hollis (1944), Freeborn (1944a,b), Sawyer (1944), Coggeshall (1943), Brown (1944), McCoy (1944a,b) and others have discussed this question. The consensus would seem to be, insofar as the present nonendemic areas of the United States are concerned, that there is no danger of the re-establishment of the disease on an endemic basis and that, while the danger of severe epidemics in these areas is not great, it would be wise to undertake certain precautionary measures to forestall or rapidly bring under control such outbreaks as may occur.

Surgeon General Parran (1944) has described the situation in the following manner:

> "Inevitably malaria cases will be dispersed through the United States by returning troops. Local outbreaks are probable in parts of the country, like the Upper Mississippi or the Hudson Valley, which have malaria mosquitoes but which have been free from infection for many years. However, it is believed that history will repeat itself and that such outbreaks will die out, because, as in the past, the environment is not favorable to perpetuating infection. But we should accelerate nature's process with mobile control forces to deal with outbreaks in any area."

To carry out these precautions in northeastern United States, a Malaria Control in War Areas sub-office has been established in the U. S. Public Health Service District No. 1 headquarters in New York City. This unit is charged with the responsibility for conducting, in collaboration with the respective state health departments and with certain other state and local agencies, the necessary protective measures around military installations in the New England states, New York, New Jersey, Pennsylvania and Delaware.

Anti-malarial measures in this area consist primarily of the following:

1. Establishment at strategic locations of mobile malaria con-
control units.

*This paper was presented at the annual meetings of the National Malaria Society in St. Louis, Missouri, 14 November 1944.

2. Extensive anopheline surveys around places where returning service personnel and prisoners of war are concentrated. Assistance to states and local agencies conducting such surveys in non-military areas, particularly where malaria is known to exist.
3. Malaria mosquito control measures where concentrations of human carriers and the mosquito vector are present.
4. Educational measures.
   a. Field demonstrations, illustrated lectures and symposia on malaria for state, district and local public health officials.
   b. The stimulation of the dissemination to practicing physicians of adequate information on the diagnosis and treatment of malaria.
   b. The training of laboratory workers in the recognition of malaria parasites and in the use of the thick film technic of diagnosis.

However, before entering into a more detailed discussion of these measures, it might be profitable to review briefly the history of malaria in the area under discussion.

That malaria was once endemic in New Jersey (Mac Donald, 1936), New York (Boyd, 1941), Massachusetts (Getting, 1944) and other parts of New England (Chapin, 1884) is clearly indicated in the literature. In 1916, the Surgeon General of the U. S. Public Health Service stated that one of the two well-recognized smaller endemic areas of malaria in this country included a section of the northern part of New Jersey, southeastern New York, Connecticut, Rhode Island and part of Massachusetts.

Getting (1944) stated that "From the study of malaria in the past and the present status of the disease in this state (Massachusetts), it can be concluded that malaria has existed in this state as an endemic disease for many years in both the eastern and western portions, that it has swept over portions of the state in at least three epidemic waves, that malaria is practically eradicated from Massachusetts and, at the present time, it is not a public health problem." He further stated that "Malaria, however, increased to epidemic proportions following the Civil War and the Spanish American War, when service men returned from malarious areas" and that an epidemic occurred in Ayer in 1918 which was due to the presence of troops at Camp Devens who were from the south.

In 1935 a local outbreak of malaria occurred in the Camden, New Jersey area which resulted in a total of about 120 cases (MacDonald, 1936).

Mobile Malaria Control Units

The Malaria Control in War Areas program has been extended to nonendemic areas in this country to prevent malaria transmission from infected military personnel to non-infected military personnel and to the civilian population. The greatest concentrations of infected individuals are found in general hospitals, prisoner of war camps, debarkation points, etc. It is in the extra-cantonment zones around such military posts that the major portion of our activities are conducted; our work complementing that of the military authorities within the boundaries of their reservations.

In addition, however, large numbers of troops are returning, permanently or temporarily, from malarious areas overseas to their home communities. It is known that the vector, *Anopheles quadrimaculatus* Say, is generally distributed throughout most of the northeastern states, often in very considerable numbers. Because of the high relapse rates and the prolonged periods of latency (Brown, 1944), such infected individuals will become potential foci for the dissemination of malaria in their communities.

Therefore, obviously, it is impossible to foretell just where outbreaks of malaria will occur. Since we cannot attempt the eradication of our mosquito vector from all communities, mobile malaria control units have been organized and distributed strategically.

Each unit consists of a station wagon and a 1½ ton truck which carries the equipment, materials and supplies necessary for entomological surveys and inspections, laryiciding, minor drainage, clearing and cleaning, and the spray-killing of adult mosquitoes. A commissioned engineer or entomologist is in charge of each unit, assisted by the necessary sub-technical and semi-skilled personnel. These units ride a predetermined circuit, spending sufficient time at each military post to perform the required control or inspection work. In addition to their routine duties, these units are also readily available, because of their mobility, for controlling such malaria outbreaks as may occur in their area.

Anopheline Surveys in Northeastern United States

During the 1943 and 1944 seasons, anopheline surveys have been conducted in 57 areas adjacent to Army, Navy, Coast Guard, War Shipping Administration and Veteran Administration establishments in all of the states comprising U. S. Public Health Service District No. 1, with the exception of Vermont. The essential purpose of these surveys was to determine the density of *A. quadrimaculatus*. All other species of *Anopheles* which were encountered were also identified and recorded. Survey work was restricted to zones of approximately a one-mile radius around each

establishment. Anopheline densities were measured principally by the inspection of a series of diurnal shelters supplemented by larval collections and, in areas which were under routine entomological surveillance, by light trap collections.

Some of the significant data relative to these surveys are presented in Table 1. These data are based upon the total number of adult resting station collections made within each state during the 1943 and 1944 seasons, exclusive of collections made in areas where control measures were being applied.

It will be noted from Table 1 that *A. quadrimaculatus* was found to be present in 32 of the 57 areas studied, and that adults of this species were found in all states surveyed, except New Hampshire. In the one area inspected in New Hampshire, larvae of *A. quadrimaculatus* were found while adults were not because of the inability to locate satisfactory stations. Moderate numbers of *A. quadrimaculatus* were taken in twelve areas in the states of New Jersey, Pennsylvania, New York, Connecticut and Massachusetts. High densities of *A. quadrimaculatus* were found in nine areas in the states of Delaware, New Jersey, New York, Rhode Island and Massachusetts. It is important to note that single station counts of *A. quadrimaculatus* were found to be as high as 200 in Massachusetts (near Taunton)[1], 500 in Rhode Island (near Wickford), 525 in Delaware (near Delaware City)[2], and 1000 in New York (near Port Byron).

It must be borne in mind that the areas where surveys were made were not selected because of previous information about anopheline abundance, nor even because of any suspicion that they were probably favorable for *A. quadrimaculatus* breeding. Such surveys were made at the request of the military authorities in specific areas where there were or were likely to be concentrations of human malaria carriers. Location of many establishments in mountainous areas, near highly developed agricultural or industrial regions, or within large cities often precluded *A. quadrimaculatus* breeding. For these reasons, it must not be concluded that the absence of *A. quadrimaculatus* from eight of the ten areas surveyed in a state such as Pennsylvania is sufficient to indicate that the species may not be present in significant numbers in other localities where local breeding conditions are more favorable. On the contrary, it is believed safe to conclude from information at hand that high densities of *A. quadrimaculatus* may be found in all of the northeastern states with

[1]On October 11, 500 females and one male *A. quadrimaculatus* were found in a single resting station near Taunton, Massachusetts. This may represent an unusual concentration for purposes of hibernation.

[2]Huffaker and Back (1943) reported an average of 4,834 females of *A. quadrimaculatus* per weekly inspection of a barn near Delaware City. These writers also reported as many as 40,000 of this species from a single inspection in this area.

the possible exception of Maine, Vermont and New Hampshire.

Table 1 also indicates that in five of the nine states studied, *A. quadrimaculatus* comprised more than half of the total anopheline population as indicated by adult resting station counts. These percentages of *A. quadrimaculatus* vary from 100 percent in Rhode Island, 98.1 percent in Delaware, and 89.3 percent in Massachusetts to 9.1 percent in Maine, 7.9 percent in Pennsylvania and 0 percent in New Hampshire.

Many persons have remarked to us that even though *A. quadrimaculatus* was present in the northeastern states, important densities were unlikely to occur until late summer because of the short breeding season. Our experience does not support this contention except in some of the most northern localities. In Massachusetts, we have noted adults of *A. quadrimaculatus* in diurnal shelters from late May until mid-October. Evidence of breeding early in the season was indicated by a collection made at Framingham, Massachusetts on June 1, 1944, in which 25 males and 15 females of *A. quadrimaculatus* were collected from under a bridge near a marsh which had been shown the previous year to breed large numbers of this species. It is generally accepted that males do not survive the winter, so it must be presumed that breeding in this area began early in May. As further evidence, we have made single resting station counts of females of *A. quadrimaculatus* as high as 500 near Wickford, Rhode Island on June 29, 1944; 1000 near Port Byron, New York on July 6, 1944; 200 near Taunton, Massachusetts on July 13, 1944, and 400 near Delaware City, Delaware on July 20, 1944.

Malaria Mosquito Control Measures

The density of *A. quadrimaculatus*, the concentration of human malaria carriers and the proximity of susceptible civilian and military personnel are taken into account when determining whether or not malaria mosquito control work shall be undertaken in an area. When such control is deemed necessary, it is performed by a mobile unit crew, or on the fixed project basis characteristic of Malaria Control in War Areas operations in the southern states.

Control measures have been restricted to the larvicidal treatment of *A. quadrimaculatus* breeding places. Clearing and cleaning have been performed where necessary as adjuncts to the larvicidal operations. Some minor drainage has been installed, particularly in situations where the construction of relatively small open earth ditches have eliminated breeding places or have considerably reduced their extent. Major drainage work has not been undertaken.

Conventional control procedures have been used. In one area airplane dusting with paris green was employed to treat the very

extensive muskrat marshes and a large wild life refuge which were breeding great numbers of *A. quadrimaculatus*. Wherever practicable, power dusters and power sprayers were utilized for effective larvicidal coverage and for the conservation of manpower, an extremely critical commodity. Prisoner of war labor was used for ditching and clearing; such labor could not be employed for larvicidal operations because too high a proportion of guards to prisoners would be necesssary.

The practice of species sanitation introduced somewhat of a public relations problem. The military personnel, general public and, to some extent, local officials used to "mosquito control" found it rather difficult to consider our work as successful when they continued to be annoyed by mosquitoes even though these were of the pestiferous *Aedes* and *Culex* species. A certain amount of missionary work had to be performed also to discourage and correct the frequently encountered impression that "anopheline" was synonymous with "malaria mosquito" and to encourage the use of the more specific *A. quadrimaculatus* or "quad".

Educational Measures

State health department personnel, district sanitary engineers, local health officers and other public health officials have spent some time in the field with our technical personnel observing and, to some extent, performing the entomological and larvicidal procedures involved in control operations. Illustrated talks and movies on the various phases of malaria control have been presented at special gatherings and at the regular meetings of state public health associations. Literature on various phases of malaria control prepared by the Malaria Control in War Areas Headquarters office, or from other sources, has been distributed.

Several fourth year medical students have been given an intensive, full-time three weeks course in this subject as part of their work in preventative medicine under Dr. H. E. Meleney. Preliminary arrangements for the training of addition medical students have been made.

Three courses of two weeks duration each were arranged for the training of laboratory technicians by Miss Aimee Wilcox in thick and thin film diagnosis. A total of 75 technicians from seven states were trained..

It is anticipated that these educational measures will be intensified during the winter months.

TABLE 1. Summary of Anopheline Surveys (Adult Resting Station Collections) Made in Northeastern United States 1943-1944

State	No. of Areas Surveyed	No. of Areas Positive for *Quadrimaculatus*	No. of Areas With Low Densities of *Quadrimaculatus* (1)	No. of Areas with Moderate Densities of Quadrimaculatus (2)	No. of Areas With High Densities of *Quadrimaculatus* (3)	Maximum *Quadrimaculatus* Count for A Single Station Inspection	Per Cent *Quadrimaculatus* From Total Anopheline Collection
Delaware	3	1	0	0	1	325	98.1
New Jersey	7	7	2	4	1	150	34.5
Pennsylvania	10	2	1	1	0	16	7.9
New York	19	13	6	5	2	100	68.5
Rhode Island	4	1	0	0	1	500	100.0
Connecticut	1	1	0	1	0	31	73.5
Massachusetts	8	6	1	1	4	200**	89.3
New Hampshire	1	0*	0	0	0	0	0.0
Maine	4	1	1	0	0	1	9.1
Vermont	0	–	–	–	–	–	–
TOTAL	57	32	11	12	9	–	–

(1) Maximum single inspection from 1 to 9.
(2) Maximum single inspection from 10 to 99.
(3) Maximum single inspection of 100 or more.
* It is of interest to note that larvae of *A. quadrimaculatus* were found in this area.
** 500 females and one male in a single station on October 11, 1944 may represent an unusual concentration for purposes of hibernation.

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A REVIEW OF EQUIPMENT USED IN APPLYING MALARIA CONTROL MEASURES ON IMPOUNDED WATERS IN THE TENNESSEE VALLEY*

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Introduction

The congressional act of 1933 creating the Tennessee Valley Authority charged it, among other things, with developing the Tennessee River for flood control, navigation, and power. This required the construction of a series of dams forming a chain of reservoirs on the main river with numerous storage reservoirs on the tributaries. The construction program has proceeded rapidly, being accelerated at the beginning of the war in order to provide increasing power for war industries and the last two reservoirs are being impounded this year. In all, the Authority is now concerned with the operation of twenty-three reservoirs, having a total water surface area of approximately 600,000 acres with 10,000 miles of shoreline. Nine of these reservoirs are located on the main river and constitute the major portion of the malaria control problem. The remaining fourteen are storage reservoirs located in relatively steep terrain of the upper valley and offer no particular malaria contol problem. Annual maintenance of limited extent is now being required in only nine of the fourteen storage reservoirs.

Malaria is not a new problem in the Tennessee River Valley. Areas of high endemicity, principally in the lower valley, are a matter of relatively recent record. From its inception, the Tennessee Valley Authority has recognized that the existing prevalence of malaria in the region might be intensified by the impoundings unless adequate control measures were devised and put into effect. The scope of the impoundages, the unfavorable character of much of the terrain and the multipurpose use of the projects combine to offer a potential problem without parallel in the country. The Tennessee Valley Authority's progressive development of malaria control practices in cooperation with the U. S. Public Health Service, the various State Health Departments and other organizations in meeting this complex problem successfully has been reported upon to this Society at intervals during the past ten years. The planning of the control measures has been accomplished by a combination of several professional specializations. Medical malariologists, biologists, and engineers have all had a part in the

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program. The application of control measures is engineering in character and has been under the direction of specially-trained sanitary engineers.

In preparing the reservoirs for impoundage and executing antilarval operations thereafter, there has been a progressive improvement and development of the equipment and facilities. It is the purpose of this paper to review the development and use of this malaria control equipment. Space will permit only the briefest summary of details but enough description is given to permit one to obtain some idea of performance and fields of application.

In brief, the reservoir preparation program has consisted of the removal of timber and other growth from the reservoirs prior to impoundage together with the drainage of depressions in the zone of water level fluctuation. The post-impoundage program is aimed at maximum utilization of naturalistic measures, most important of which is the coordinated use of desirable water level management and shoreline maintenance practices. Where these naturalistic measures are not fully effective, larvicides are used to provide the necessary degree of mosquito control.

House mosquito-proofing has been used as a supplementary malaria control measure in a few of the most serious mosquito problem areas of the Wheeler, Guntersville, and Kentucky Reservoirs. More recently in the Kentucky Reservoir, the program of reservoir preparation has included permanent shoreline improvement measures such as diking and dewatering, or filling and deepening to eliminate mosquito breeding areas, or removal of families within mosquito flight range where this measure appeared suitable and most economical. It is planned to expand the permanent improvement program to embrace areas in other reservoirs on the main river already impounded.

Reservoir Preparation

The reservoir preparation work has been executed by the Reservoir Clearance Division headed by Mr. H. E. Davis, a veteran of long experience in reservoir preparation antedating his period of service with the Tennessee Valley Authority. Mr. Davis and his associates have done much to advance reservoir preparation practices through use of machines, and this paper would not be complete without some mention of them. Adequate reservoir preparation is considered basic to the subsequent effective and economical application of post-impoundage mosquito control measures.

In reservoir clearance work, felling trees has been an ax and cross-cut saw operation, though several types of engine-powered saws have been investigated and one or two tried without much success. Earlier it was the practice to remove the tree limbs and cut the trunks into convenient lengths, piling the brush and logs

by hand or team for subsequent burning. More recently, under the stress of completing the clearing in the Kentucky Reservoir on schedule under wartime labor conditions, a caterpillar timber rake was developed which exceeded all expectations and reduced the clearing cost appreciably in this reservoir. The machine is a Caterpillar D-Eight Bulldozer with teeth attached to the bottom edge of the blade and with a floating device on the bottom of the blade to keep it from plowing into the ground (Fig. 1). Reasonably low stumping is required for best use of this machine. In operation the timber is felled, preferably in one direction, and pushed into windrows for burning. After a short period for drying, fire is applied. When the windrows have burned down, a caterpillar D-Six rake constructed similarly to the D-Eight is used for pushing together unconsumed chunks and logs until burning is complete. In average timber in the Kentucky Reservoir, this machine with one operator and a flagman or oiler piled approximately seven acres of felled timber per day.

Fig. 1. Caterpillar timber rake

A special bank machine consisting of a caterpillar tractor with hoisting drum and cable running over an attached "A" frame has filled a special need in pulling out trees which were necessarily felled into the water along the steep banks of major streams (Fig. 2). On account of mobility, a small caterpillar tractor with winch has proved quite serviceable on bank work as a supplementary machine.

Cutting and piling by hand and teams is necessary where the timber rake is not used. Under average conditions, approximately

132 man hours of labor on flat clearing and 180 man hours on bank work are required per acre on this type of work.

Fig. 2. Bank Machine

In compliance with State Health Department regulations, the practice has been to connect marginal depressions with the main body of the reservoir. Open ditches, largely constructed with a one-half yard dragline, have been used for this purpose. Under average conditions in the Kentucky Reservoir, these machines moved around 200 to 300 cubic yards of earth per day. A one-half yard dragline is preferred for this work since larger sizes would be difficult to move from one project to another and smaller sizes would not be efficient in constructing the larger ditches. These machines are used principally in removing timber and draining marginal depressions prior to impoundage.

In the large reservoirs, it is necessary to begin clearing two or three years in advance of filling. This allows a secondary growth of sprouts and plants which require removal around the margins just prior to impoundage. Final conditioning of the margins is usually done in the fall with filling taking place during the ensuing winter. Earlier it was the practice to remove this growth by hand, but in recent years the Authority has developed the use of machines for cutting and piling the material preparatory to burning. Initially, the machine operation is preceded by the use of running fires where all of the standing material possible is consumed. The remaining material is cut preferably through use of team-drawn or motorized agricultural mowers. In average marginal growth, approx-

imately four acres can be mowed by team units and ten by tractor units per day. One operator is used as a rule though occasionally a helper is required in extremely heavy cutting. Where the area contains sprouts of a size which cannot be cut with mowers, it is necessary to supplement the mowing with hand cutting and a number of useful hand tools have been developed for the purpose including the "Pat Miller topping blade" made from old cross-cut saws. Hand cutting and burning requires about two to three man days per acre. Agricultural rakes are normally used for windrowing the material prior to burning. One rake and operator will cover ten to twelve aces per day.

Recently, in the Kentucky Reservoir, the Reservoir Clearance Division developed what may be termed a brushing harrow which is pulled by an ordinary crawler-type tractor (Fig. 3). This machine is used to knock down or break up the heaviest growth of sprouts and vines and is followed by a clean-up operation performed by hand. The machine was designed principally to reduce man hours during a critical labor shortage but it also promises to reduce overall costs. In the normal heavy marginal growth of the Kentucky Reservoir, this machine would cover about eight acres per day. The use of this harrow reduced the labor cost about 50 per cent.

Fig. 3. Tractor-drawn brushing harrow

Shoreline Maintenance

In removing annual marginal growth after impoundage, several of the same machines are used as on pre-impoundage preparation. These are the mowers and rakes and small tools for removal of marginal growth. In addition, a floating underwater weed cutter is employed where "repeat-cutting" is indicated in the control of such deep-rooted aquatics as lotus and cowlily. It has also been used with success in cutting deeply-submerged sprouts and stiff-stemmed annuals. Figure 4 shows the underwater weed cutter used on this work. In average growth, it will cover about four acres per day. One operator and a standby assistant are required. This machine has had quite a wide use over the country on aquatic growth control operations.

Fig. 4. Underwater weed cutter

The Authority has made rather extensive studies on the use of herbicides in the control of marginal plants. The most extensive use has been made of petroleum oil (kerosene, diesel or fuel oil) in the control of alligator grass, a particularly objectionable exotic plant which became established in Wilson Reservoir and spread into the Pickwick Reservoir immediately below. Control consists of saturating the plant and ground with oil. For this purpose, a motor-powered pressure spray pump is used which may be mounted in a boat or truck according to needs and the same pumping unit is used as employed in applying oil as a larvacide by boat. Knapsacks are used to treat areas beyond reach of the spray hose. A very effective arboricidal treatment has been developed for such objectionable

coppice as black willow. It consists of thoroughly applying a spray of one of the oils mentioned above to the face and edges of the low cut stump with an ordinary knapsack spray can.

In the annual growth removal operations along the margins of the reservoirs, normally executed during the fall of the year, running fires are employed to the maximum both for reasons of economy and for the killing effect on the seedlings of woody plants. A knapsack weed burner, of which there are several on the market, has been found advantageous in firing the growth as well as brush and log piles.

Larvicides

Larvicides are used on the Authority's impoundages as necessary for limiting *Anopheles quadrimaculatus* production under circumstances where water level management and other antilarval measures cannot be made effective. Airplane dusting has received the most extensive use since it is a method particularly suited to rapid application in these situations. For this work the Authority uses its own pilots and equipment consisting of five units which operate in the nine reservoirs on the main river. Model 4-DX Stearman biplanes are specially outfitted for dusting. Full details on the Authority's airplane dusting will be found in a report presented at the 1943 meeting of the National Malaria Society.* Recently, on certain experimental work, this airplane was outfitted quite readily for the experimental application of thermal aerosols and oil sprays.

Some experimental dusting was done with a cub airplane this year to determine the usefulness of a light airplane in this method of applying larvicides. The airplane used was outfitted with dusting equipment designed by the U. S. Department of Agriculture. The experiment demonstrated the need for some mechanical improvements in the equipment and indicated possible economies from using a light airplane where breeding situations exist as numerous small areas; however, the limitations of an airplane of low horsepower and small load-carrying capacity were evident.

Boat larvicidal units are used for treating areas which cannot be reached with the airplane for reasons either of economy or of inaccessibility. Obviously, a narrow strip of breeding area lying along a steep and tortuous shoreline can best be treated by boat. For such situations the water-oil method is employed where the larvicidal oil is carried to the point of application by a water stream provided by engine-powered pump taking suction from the lake.

*Kruse', C. W., A. D. Hess, and R. L. Metcalf, 1944. Airplane Dusting for the Control of *Anopheles quadrimaculatus* on Impounded Waters. Jour. Nat. Mal. Soc., 3: 197-209.

Both inboard and outboard powered equipment is in use and there is perhaps a field for both types of units. Recent trends are toward the outboard units due to their lightness, low draft, and better manueverability. However, the inboard units have the most reliable power and are especially adapted to the supplementary use of transporting men, tools, and equipment on shoreline maintenance work. Both units require two operators, and under average conditions in the Authority's reservoirs will treat eight to ten miles of shoreline per day of intermittent breeding situations.

More recently in the experimental trial of a larvicidal oil requiring very low application rates, one of our standard inboard water-oil units was modified to provide a "sight-feed" and small calibrated needle valve used in regulating the dosage. For routine larvae dipping and inspection, a light, fast outboard equipped with two outboard motors has been provided. A 15 or 22 horsepower is used on long straight-a-way runs, and a ten horsepower 360° revolving motor is used when the boat is operated close to shore on inspection work.

Special Spray Equipment

During the past year it was necessary to design equipment and plan an operation for applying pyrethrum insecticidal spray in houses of the lower Kentucky Reservoir during an emergency expected to develop with summer impoundage of the reservoir. Consultation was had with Mr. F. W. Knipe, Sanitary Engineer with the Rockefeller Foundation, who had been associated with the house spraying work in India. Under the conditions in Kentucky Reservoir, it was decided to use a dry ice pressure spray method, and equipment was designed for this purpose (Illustrated in Fig. 5 with detailed plans in Fig. 7). It is made up of standard parts available on the open market. The dry ice container is a standard two and one-half gallon underwriter approved fire extinguisher tank. The spray gun is of the pressure feed type with modified nozzle. Full details and parts list are available on request. Twelve units were assembled for covering an area of about 1200 houses at weekly intervals in the Kentucky Reservoir. While the impoundage was delayed until late summer, and for this reason the emergency was not nearly so serious as anticipated, this dry ice pyrethrum spray unit was given fairly extensive trial in both the Kentucky and Wheeler Reservoirs. It fully measured up to expectations. After an initial round when considerable time was required to explain the purpose of the work to householders, two operators in a pick-up truck could spray 40 to 50 rural houses per day. Two charges or about 50 lbs. of dry ice were required per day for each unit.

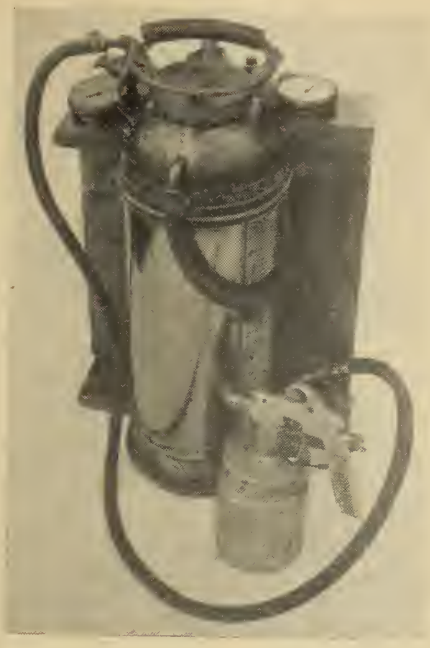

Fig. 5. Dry ice pressure sprayer

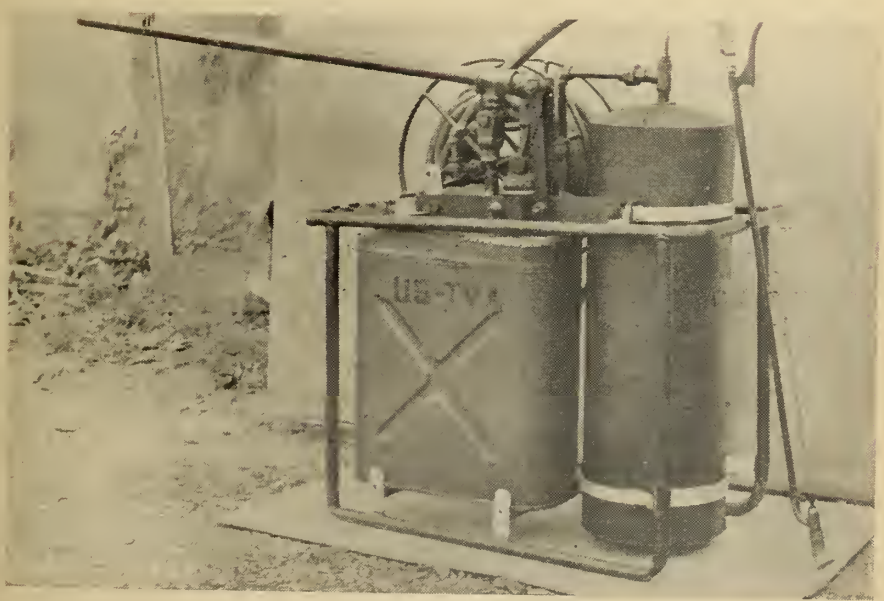

Fig. 6. Hand-operated pressude sprayer

Between one and two pints of spray containing 0.1 per cent pyrethrins were used per house though this was in excess of the ¾ ounce per 1000 cubic feet of volume recommended to kill *A. quadrimaculatus* adults.

Another fairly simple spray unit was assembled during the past summer for the experimental application of straight insecticidal spray (Illustrated in Fig. 6 with detailed plans in Fig. 8). It provides a fairly coarse spray through a standard whirl plate agricultural nozzle with .04" diameter orifice under 100# pressure. The insecticide is held in a five gallon open container from which a high pressure hand-operated pump takes fluid by suction with delivery into the spray line to which is connected a five gallon air-cushion tank. A very uniform spray is produced with an occasional stroke of the pump by the operator. Two or three spray lines could be easily operated from this unit. The commercial agricultural spray manufacturers can supply an approximate dulpicate. Two men operating in a pick-up truck can treat about 25 houses per day.

Miscellaneous Equipment

For general utility transportation, two types of boats are in use, one a commercial launch of 18' to 20' length, and the other a 24' shop-made unit of special design having a rugged propeller guard, flat bottom, and a prow which facilitates frequent landing along the shoreline.

One central dust mixing plant at Wilson Dam serves the Valley. The unit used is an electric motor-powered batch mixer which was specially equipped with an oversized exhaust fan to limit the dust hazard.

Malaria control bases of operation are required at various points in the reservoirs. They usually consist of a storehouse, dock, oil and gasoline tanks, a well, and sanitary facilities. The latest feature in the Kentucky Reservoir provides a floating storehouse for greater convenience in transferring equipment to and from the boats.

Permanent Shoreline Improvement

It might be well to touch briefly on the equipment used on permanent shoreline improvement in the Kentucky Reservoir and which it is hoped can be expanded to embrace the most serious problem areas in other main stream reservoirs.

The dikes on the eight dewatering projects were constructed with large draglines and the slopes were leveled with bulldozers. The interior drainage was constructed with one-half yard draglines. The pumping stations are of reinforced concrete. The full automatic pumps are low head high volume type and are powered by either electric motor or gasoline engine depending on the pump station locations with respect to power lines. Starting and stopping is automatic. There are nine major filling projects in the Kentucky

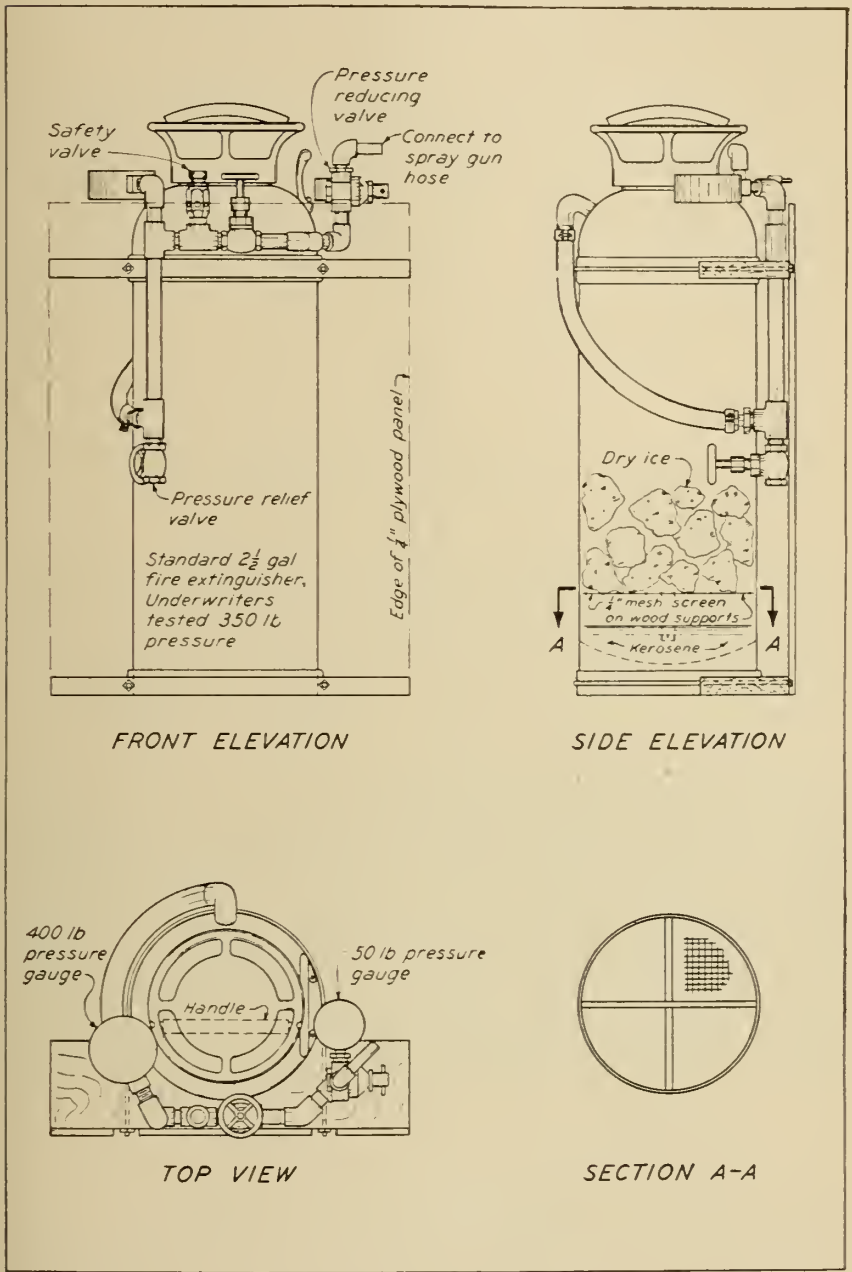

Fig. 7. Plans of dry ice pressure sprayer

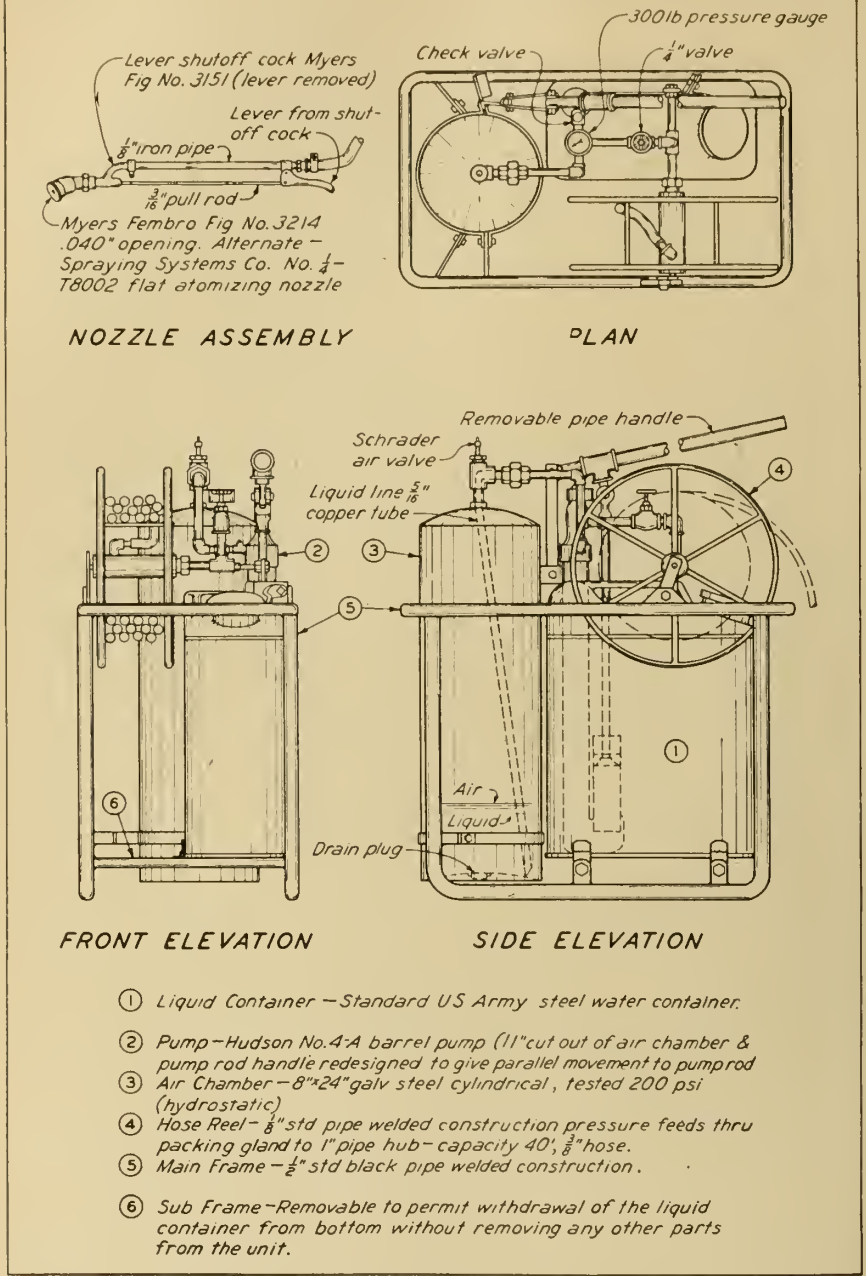

Fig. 8. *Plans of hand-operated pressure sprayer*

Reservoir. A three-foot growth zone below the normal maximum summer elevation was assumed on the basis of observations as to the extent of growth invasion in other main river reservoirs. The general plan was to excavate the lower half of this zone, placing the dirt on the upper half, thus eliminating the problem zone through, what is termed, the "cut-and-fill" combination. Where cut and fill can be balanced, which is not always the case, the yardage required to be moved is just one-fourth that required for complete filling or deepening. Conventional land earth moving and leveling equipment consisting of LeTourneaus and bulldozers were used on this work (Fig. 9). Stumps in the cut areas were dynamited, pushed into piles with bulldozers, and burned. A few boggy situations were encountered which interfered considerably with the operations, necessitating use of draglines for casting material to where it could be handled by bulldozers. It was necessary to limit the work to the dry season of the year, normally June 1-December 1. The machines on this operation averaged 400 to 500 cubic yards per shift of eight hours.

Fig. 9. Bulldozer and LeTourneau grader

The advanced engineering planning on the permanent shoreline improvement work was directed by Mr. C. W. Okey of the Water Control Planning Department. The construction work was executed by the Construction and Maintenance Division headed by Mr. T. D. Lebby. The success of the work may be attributed in no small measure to the ability and cooperation of these men and their associates.

Summary

A brief statement is given of the impounded water development in the Tennessee River Valley and the associated malaria control problem in a region where areas of high endemicity are a matter of relatively recent record.

The malaria control practices on the impoundages were developed through cooperation with state and national health organizations. In brief, the malaria control program has consisted of the removal of timber and other growth from the reservoirs prior to impoundage together with drainage of depressions in the zone of water level fluctuation and, after impoundage, the periodic removal of objectionable marginal vegetation and the supplementary use of larvicides, insecticides, and house mosquito-proofing in selected areas. Permanent shoreline improvement was used in the Kentucky Reservoir and plans are being made to apply this measure to problem areas of other main stream reservoirs already impounded.

A brief description is given of the various types of machines, equipment and facilities which have been developed for executing malaria control measures on the impoundage.

THE DETECTION OF THE PLASMODIA OF HUMAN MALARIA IN BLOOD FILMS BY FLUORESCENCE MICROSCOPY*

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(Received for Publication 14 November 1944)

Introduction

Fluorescence microscopy has recently been successfully applied to the routine identification of *Mycobacterium tuberculosis* in sputum smears (Hagemann 1938, Richards et al, 1941). Bock and Oesterlin (1939) reported good visualization of *Plasmodium knowlesi, P. ovale,* and *P. vivax,* using several fluorescent stains; however, no attempt was made to develop the method for the routine diagnosis of malaria. Patton and Metcalf (1943) reported on the fluorescence microscopic visualization of two species of bird malaria parasites using a number of fluorochromes. Because of the striking contrasts obtained and the clarity with which the parasites could be detected, it was thought that the utilization of this method in the routine diagnosis of malaria might result in an increase in the efficiency of examination of blood smears. This paper presents the results of a detailed study of the applicability of the fluorescence method to the diagnosis of human malaria.

Equipment

The methods of fluorescence microscopy employed have been described in detail by Metcalf and Patton (1944). A short resume' is presented here for the sake of clarity. The equipment consists of a standard monocular microscope with Abbe' condenser and mechanical stage, a yellow glass filter (equivalent to Wratten K_2) ground to fit at the ocular diaphragm, a high-pressure mercury vapor lamp, G. E. type H-4, with its ballast transformer, and two Corning glass filters (2 inch polished squares), #5840 red-ultra and #4308 light shade blue green. The mercury vapor lamp is placed in a standard microscope lamp housing fitted with reflector and condensing lenses, and the two filters are placed in the filter holder of the lamp housing (Fig. 1).

The two Corning filters serve to isolate that portion of the ultra-violet spectrum lying between 350 and 400 millimicrons, while the yellow ocular filter absorbs stray ultra-violet radiation from the fluorescent light entering the eye.

*This paper was presented at the annual meetings of the National Malaria Society in St. Louis, Missouri, 15 November 1944.

Fig. 1. Equipment for Fluorescence Microscopy — microscope, lamp housing, transformer, eyepiece filter, corning filters for lamp, mercury vapor lamp.

Methods

The blood films used in this study were routine preparations taken from patients undergoing malaria fever-therapy.[1] They are prepared for examination as follows:

1. Fix thin films in absolute methyl alcohol for 5 minutes and dry.
2. Stain in aqueous 0.1% solution of berberine sulfate[2] or rivanol[3] (2-ethoxy, 6, 9-diaminoacridine lactate) for 10 minutes.
3. Wash in distilled water for 10 to 30 seconds and dry. The relative intensity of the background can be controlled by the length of the washing period and this should be adjusted to give the best contrast obtainable.

The slides are then examined by the fluorescence technique. The only critical point in the miscropic examination is the adjustment of the positions of the lamp and the substage condenser to give the most intense concentration of the ultra-violent beam. This occurs when the image of the lamp arc is formed in the plane of the blood film. The concave side of the microscope mirror is most suitable.

[1]Furnished by Dr. R. B. Watson, Tennessee Valley Authority, Health and Safety Department, Memphis, Tennessee to whom the writer is indebted for valuable advice as to the course of these studies.
[2]Merck and Company.
[3]H. A. Metz and Company.

Results

Thin Films

The results obtained with thin films have been very satisfactory. A number of fluorochromes have been tried with the results indicated in Table 1. Of these, rivanol and berberine sulfate have given by far the best results. Several hundred slides of the three common species of human malaria, *Plasmodium vivax, P. falciparum,* and *P. malariae,* have been studied. In all cases the parasites appeared as bright luminous bodies against a dark background (Figs. 2 and 3). With rivanol and berberine sulfate fluorochromes,

TABLE 1.

Effect of Staining Thin Films of Human Plasmodia with Aqueous Fluorochrome Solutions

Fluorochrome	Fluorescent Appearance			
	Plasmodia	Leucocytes	Erythrocytes	Effectiveness
Berberine Sulfate	bright yellow	bright yellow	dark	++++
Rivanol	gold yellow	yellow	dark	++++
Thioflavin	golden	light yellow-green	dark	+++
Coriphosphine O	dull orange	bright orange faint yellow membrane	dark	+++
Acridine Yellow	yellow	yellow orange	dark	++
Acridine Orange	dull orange	deep orange	dark	++
Auramin O	yellowish	yellow	dark	++
Atabrine	faint yellow	faint yellow	dark	+
Acriflavin	very faint	faint orange nuclei, yellow cytoplasm	dark	−
Phosphine 3 R	uncertain	faint orange	dark	−
Primulin Yellow	blue	red-orange nuclei, bright yellow dots in cytoplasm	olive-green	−
Titan Yellow	dull green	bright yellow cytoplasm	orange	−
Eosin	−	pink nuclei	dull brown	−
Congo Red	−	red nuclei	dull orange	−

the parasites could be readily distinguished using a 44x objective and 10x ocular, but best results were obtained using an immersion lens (97x) and a 5x ocular. A 10x ocular may be used, but it slightly decreases the light intensity. Crown immersion oil which is practically non-fluorescent is used as an immersion fluid in place of cedar wood oil which is highly fluorescent.

The appearance of the three species of parasites is much like those seen in Giemsa preparations with the exception of color differences: however, *P. vivax* does not show anything analogous to Schüffner's dots. *P. malariae* is characterized by a large amount of black, non-fluorescent pigment.

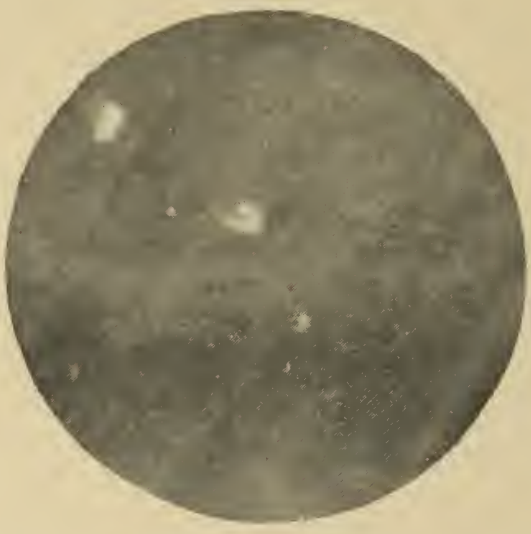

Fig. 2. Appearance of P. vivax and leucoyte stained with bererbine sufate, under fluorecence microscope — 900x.

All stages of the parasites stain equally well and, in general, appear as follows:

Trophozoites—Appear as sharp yellow rings with brighter yellow chromatin dots.

Schizonts —Appear as bright yellow discs enclosing irregular areas of dark (non-fluorescent) pigment. Segmenters show distinctly segmented appearance.

Gametocytes —Appear as yellow irregular-shaped bodies with brighter chromatin material, enclosing dark pigmentation.

Due to the darkfield obtained, the luminous parasites are very readily detected by reason of their contrast to the dark non-fluorescent erythrocytes. Several persons totally unfamiliar with diagnostic procedure were briefly instructed in the use of the method and were able to readily distinguish plasmodia.

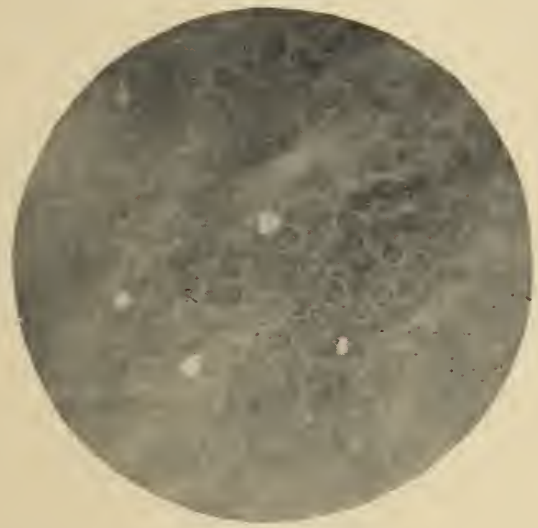

Fig. 3. Appearance of P. malariae and blood platelets stained with rivanol under fluorescence microscope — 900x.

To compare the efficiency of the fluorescence technique with the ordinary Giemsa method, three smears of *P. vivax* and one each of *P. malariae* and *P. falciparum* were divided longitudinally with a wax pencil line. One-half of each slide was stained with Giesma and the other half with berberine sulfate. Parasite counts were then made in an equal number of fields on each half at 970x magnification. The results obtained were as follows:

	No. Fields Counted	No. Parasites Giemsa	No. Parasites Fluorescent
P. vivax	150	72	65
P. malariae	100	27	28
P. falciparum	100	7	6

Buffered Staining—In order to ascertain the effect of the pH of the staining medium on the relative staining intensities of parasites, leucocytes, and erythrocytes, experiments were undertaken with staining solutions buffered by HCl, NH_4OH, and mixtures of KH_2PO_4 and Na_2HPO_4.

It is apparent from the data which are given in Table 2 that maximum contrast between malaria parasites and erythrocytes is obtained at or near neutrality. The pH of an aqueous 0.1 solution of rivanol in distilled water was approximately 7.0. Therefore, the use of a buffered staining solution is not necessary. Buffering at neutrality, however, might be of value where a large number of slides are to be stained with a given volume of solution. Where solutions of rivanol are to be kept for long periods, a trace of phenol should be added to prevent the growth of mold.

TABLE 2. Relative Staining Intensities at Varying Hydrogen Ion Concentrations.

Stain	pH (glass electrode)	Parasites	Erythrocytes	Leucocytes	Effectiveness
Rivanol 0.1% in 1/10 N HCl	1.0	dull yellow	–	nuclei-dull yellow	+
Rivanol 0.1% in phosphate buffer	4.5	yellow	–	nuclei-yellow	++
Rivanol 0.1% in phosphate buffer	6.9	golden	–	nuclei-yellow	++++
Rivanol 0.1% in distilled water	7.0-7.2	golden	–	nuclei-yellow	++++
Rivanol 0.1% in 1/30 N NH_4OH	9.8	orange-yellow	olive green	medeii bright yellow capsule-olive green	+++
Rivanol 0.1% in 1/10 N NH_4OH	11.2	only chromatin material visible	yellow green	yellow	+

Counterstaining—An attempt was made to counterstain the leucocytes with Congo red, eosin, and methylene blue. It was hoped that this might decrease the fluorescence of the leucocytes so that parasites could be detected as bright spots at a very low magnification. All the attempts, however, while resulting in some fading of the leucocyte fluorescence, also decreased the intensity of the fluorescence of the parasites.

Thick Films

Dehemoglobinization of the thick films for 15 minutes in tap water, followed by staining with rivanol or berberine sulfate as outlined for thin films, gives adequate staining of plasmodia but the residuum of erythrocytes left on the slides retains enough of the fluorochrome to reduce the contrast between parasite and darkfield and thus makes identification more difficult. This can be largely obviated by immersing the thick film in 0.1% hydrochloric acid

solution for 15 minutes, followed by washing briefly in distilled water and then staining in 0.1% aqueous fluorochrome. By this procedure almost all the hematin is removed from the slide and the contrast between parasite and background is greatly improved giving a very satisfactory preparation. Following staining the slides should be washed in tap water for 2 to 3 minutes. The morphological appearance of the parasites in the thick films is very similar to that occurring in Giemsa stained smears.

Discussion

The employment of fluorescent dyes as histological stains which are then irradiated by ultra-violet rays produces, in effect, a light source in the microscopic object itself. This results in the most perfect condition for image formation in microscopy in that refractive difficulties inherent in condensing lenses are eliminated. This fact, coupled with the darkfield produced, results in extreme sharpness and clarity of observation.

As applied to examination of plasmodia in blood films, the method appears to be of interest in routine examination. As the parasites appear as luminous bodies within the dark outlines of erythrocytes, they can be readily observed even in rapid scanning of the slide.

Summary

A method is presented for the fluorescent microscopic identification of the plasmodia of human malaria in blood films. Of fourteen fluorochromes studied, berberine sulfate and rivanol gave the best visualization of plasmodia with the fluorescent microscope. It is thought that, because of its simplicity, this method might be of value in routine malaria surveys.

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CALL FOR THE 28th ANNUAL MEETING

To the Members of the National Malaria Society:

Official notice is hereby given to the members of the National Malaria Society that the 28th annual meeting of the society will be held in Cincinnati, Ohio, from November 13th to 15th inclusive, conjointly with that of the Southern Medical Association. Two scientific consessions are planned on the 13th and 14th, and a business session for the 15th. All sessions are to be held at the GIBSON HOTEL which is also designated as headquarters for the society. Members who expect to attend are urged to immediately make application for reservations to:

Dr. Robert Biltz, Chairman
Hotel Committee, Southern Medical Association
910 Dixie Terminal Building
Cincinnati 2, Ohio.

Space on the program is filling rapidly, but additional papers are acceptable. Presentation time will be limited to 15 minutes. Committee chairman should have their reports in readiness for the business session.

MARK F. BOYD
Secretary

DESCRIPTION OF *ANOPHELES (NYSSORHYNCHUS) DUNHAMI*, A NEW SPECIES FROM THE UPPER AMAZON BASIN*

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Servico Especial de Saude Publica
Belem, Pará, Brazil

(Received for publication 24 February 1945)

A new species of Anopheles belonging to the so-called "tarsimaculatus complex" of the *Nyssorhynchus* group of mosquitoes has been captured in large numbers on animal bait in Tefé, Amazonas, Brazil. In egg, larval and adult characteristics this mosquito closely resembles *Anopheles goeldii* Rozeboom and Gabaldon, 1941. The genitalic characteristics distinguish it from both *Anopheles goeldii* and *Anopheles nunez-tovari*, Gabaldon, 1940. Specimens were sent to Dr. Gabaldon, author of *nunez-tovari*, and co-author of *goeldii*, for comparison with his species. In a personal communication he concurs in the opinion that the mosquitoes from Tefé differ from both of these and constitute a new species. Its relation to malaria is not known, although it is not suspected of being a vector as the region from which it was collected shows one of the lowest malaria rates of any area studied in the Amazon Valley. For this mosquito the name *Anopheles (Nyssorhynchus) dunhami* is proposed in honor of General George Dunham, U. S. A., Assistant Coordinator of Inter-American Affairs.

The type specimens have been forwarded to the National Museum in Washington, D. C.

Adult female. Head: vertex with about twelve long white hair-like setae directed forward, interspersed with short recumbent white scales, remaining dorsal portion covered with erect white spatulate scales except for short black scales on posterior margin. Antennae with scattered white scales on torus and first flagellar segment; all segments sparsely clothed with few long and numerous short white hairs. Palpi: first segment all dark; second and third segments mainly dark, with few light scales apically on second and scattered white scales centrally with narrow white apical ring on third; fourth segment mainly white with basal black ring and ventral line of dark scales; fifth segment white with basal black ring. Proboscis all dark.

Thorax: integument of mesonotum gravish brown with dark bare spot on pre-scutellar space, and small dark bare spot at each side of middle portion. Vestiture of scattered long dark hairs and

*The studies herewith reported were part of the program of the *Servico Especial de Saude Publica* maintained jointly by the Ministry of Education and Health of Brazil and the Coordinator of Inter-American Affairs.

whitish scales, small recumbent except at margins and wing base where scales are larger, narrow and semi-erect; scutellum covered with whitish scales and long dark hairs; sternopleura with patch of white scales on upper portion. Knobs of halters with small pale scales.

Legs: coxae and trochanters with patches of white scales, femora and tibia dark with scattered white scales on outer portion, mainly pale on inner portion. Front tarsi: segments one and two mainly dark with apical white ring; segment three basal half black, apical half white; segment four all dark; segment five basal half black, apical half white. Mid tarsi: segment one dark on outer portion, light on inner with apical white ring; segment two dark with apical white ring; segments three and four mainly dark with few light scales apically; segment five dark basally, light apically. Hind tarsi: segment one mainly dark with line of light scales and few white scales apically; segment two with basal 22 to 40 percent black, remainder white; segments three and four all white; segment five black on basal half, light an apical half.

Wing: Costa: B1 broad; B2 either subequal to or much broader than preceding black spot; B3 smaller than B2; M1 and M2 moderate; Sc and Ap moderate. Subcosta: M1 and M2 large, extreme tip with few white scales. First vein: base of stem pale, M1 and M2 larger than on costa, Sc and Ap larger than on costa. Second vein: apical half of stem mainly pale with two small black spots, upper branch with small pale spot at base and tip, pale large spot corresponding to Ap, lower branch with three pale and two black spots. Third vein: predominantly pale with black spot near each extremity. Fourth vein: stem mainly dark with preapical pale spot; upper branch with small pale spot at each extremity and another at middle; lower branch dark with narrow apical spots. Fifth vein: stem pale with subapical dark spot; upper branch predominantly pale with two black spots on basal half and one subapical black spot, lower branch pale with subapical black spot. Sixth vein: predominantly pale with black spot near each extremity.

Abdomen: integument dark brown. Tergite one without scales, succeeding tergites with progressively more yellowish scales. All segments except first with lateral tufts of dark outstanding scales. Cerci densely covered with yellowish scales. Sternite one without scales, succeeding six sternites with preapical pale, and apical black scales.

Male Terminalia. Fused dorsal lobe of claspette similar to *Anopheles goeldii* (Plate I, figs. 1, 3); apex wide with shallow, broad excavation. Preapical plate small and lightly chitinized. Hairs on basal lobule short. Mesosome short, wide and without leaflets;

Fig. 1—*Fused dorsal lobes of claspette of A. dunhami.*
Fig. 2—*Mesosome of A. dunhami.*

Fig. 3—*Fused dorsal lobes of claspette of A. goeldii.*
Fig. 4. *Mesosome of A. goeldii*

Fig. 5—*Eggs of A. dunhami.*

Plate 1

membraneous tip wider than long (Plate I, Fig. 2). Easily differentiated from *Anopheles goeldii,* mesosome of which is long, narrow usually with leaflets, and with membraneous tip longer than wide (Plate I, fig. 4).

Egg. Egg similar to that of *Anopheles goeldii.* Floats widely separated with about 25 ridges. Frill wide at both ends extending from floats around each end of egg. (Plate I, fig 5).

Larva. Anterior clypeal hairs single with few delicate branchlets visible only under high magnification. Inner clypeal space slightly smaller than outer space. Posterior clypeal hairs, long, unbranched. Antennal hairs inserted on basal fourth of antennal shaft, short, with four to six branches.

Submedian prothoracic group of hairs widely separated. Inner hairs palmate with 9-10 leaflets on small lightly chitinized base; median hair large inserted on well chitinized base; outer hair small. Palmate hairs on metathorax and on first abdominal segment poorly developed; palmate hairs on other abdominal segments large with numerous pointed leaflets.

Summary

A new species, *Anopheles (Nyssorhynchus) dunhami,* is described from Tefé, Amazonas, Brazil, in a region where malaria is not prevalent. By egg, larva and adult color pattern it is similar to*Anopheles goeldii.* The male terminali distinguish it from both *Anopheles goeldii* and *Anophele nunez-tovari.*

DIFFERENTIATING THE LARVAE OF *ANOPHELES GEORGIANUS* KING, *A. BRADLEYI* KING, AND *A. PUNCTIPENNIS* (SAY)

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(Received for publication 18 May 1945)

While the larvae of *Anopheles georgianus* King and *A. bradleyi* King are usually distinguishable on the presence or absence of functional palmate hairs on abdominal segments 3 and 7, these are sometimes missing or otherwise confusing and a separation of these species is dependent upon other differential characters. Those set forth by King (1939) are as follows:

	georgianus	*bradleyi*
Inner clypeal hairs	closely approximated basally, the tubercles separated by less than the diameter of one of the basal tubercles.	usually closely approximated but sometimes separated by more than the diameter of one of the basal tubercles.
Posterior clypeal hairs	simple or forked at middle or apical third, occasionally 3-branched at tip.	usually simple, occasionally split on apical half.
Palmate hairs	well developed only on segments 4, 5 and 6, those on segments 3 and 7 slightly differentiated but apparently not funtional.	developed on segments 3 to 7 inclusive, those on 3 and 7 somewhat smaller than the others and most of the leaflets with smooth margins.
Antepalmate hairs (hair 2) on segments 4 and 5	with 2 to 6 branches (about 50 per cent with 3 branches, 24 per cent with 4 or more).	usually simple or double, very rarely triple.
Lateral hair on segment 4	with 3 to 6 branches, usually 4 or 5, and usually with comparatively long basal stalk or irregular branching.	usually branched from near base, sometimes with secondary branching farther out.
Lateral hair on segment 5	with 2 to 4 branches, usually arising from the same point near the base.	same as for segment 4.

These characters are helpful in separating *georgianus* and *bradleyi* but due to the variations and overlapping of these differences there is a need for more consistent differential characters for separating them.

The larvae of *A. bradleyi* and *A. punctipennis* (Say) are often difficult to separate due to their close similarity. King, Bradley and McNeel (1944) give the following distinguishing characters:

bradleyi

Leaflets of palmate hairs on segments 3 and 7 slender, usually somewhat smaller than those on segments 4 to 6 and mostly with smooth margins. Antepalmate hairs on segments 4 and 5 single or double; distance between clypeal hairs variable.

punctipennis

Palmate hairs on segment 3 with broad leaflets, usually notched or serrated on outer half and about equal in size to those on segments 4 to 6. Antepalmate hairs on segments 4 and 5 usually double, except in specimens from central Florida, in which they are usually single.

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For distinguishing *bradleyi* from *punctipennis,* Matheson (1944) states "the only differentiating characters are that the palmate tufts of abdominal segments 3 and 7 are smaller than the others" and, for distinguishing *georgianus,* "palmate tufts are well developed only on segments 4 to 6; antepalmate hairs (hair No. 2) have only 2 to 5 branches."

It is evident that additional differential characters are needed for distinguishing the larvae of these anophelines.

In the present study of differences and character variations in the three species, a series of mounted 4th instar larvae were examined consisting of:

(1) 55 *A. georgianus* from 19 localities in the Atlantic and Gulf coastal states from North Carolina to Louisiana inclusive, collected during the period March 1942 through March 1945 with dates representing each month of the year.

(2) 53 *A. bradleyi* from 10 localities in the coastal states from Virginia to Texas inclusive, excepting North Carolina and Louisiana, collected during the period February 1938 through February 1945 and representing all months except January, August and November.

(3) 58 *A. punctipennis* from 17 localities in 7 southeastern states and Washington, D. C., and representing the months May through November.

The following characters were found to be a useful supplement to the palmate hair character in separating *georgianus* and *bradleyi*:

	georgianus	*bradleyi*
Antennal spicules	fine and slender with little thickening at base and not pigmented.	course and stout, thickened at base and darkened by pigmentation or sclerotization.
Head hair 9 or outer occipital hair	rarely reaching beyond bases of frontal row of hairs and little if any longer than inner occipital hairs.	usually reaching beyond bases of frontal hairs and distinctly longer than inner occipital hairs.
Hair 5 of abdominal segment I, or first hair dorsal to the lateral hair	5 to 7 branches of variable length arising irregularly and with secondary branching outward from base.	4 or 5 long branches arising from near the base, occasionally with the central branch divided or with a single branch arising outwardly.
Prothoracic hair 1 on the inner submedian prothoracic hair	usually with 3 to 5 branches arising variously along central portion.	simple (about 30 per cent) or weakly branched at the tip.

These are listed in the order of their dependability as differential characters. The antennal spicules of all *georgianus* were definitely more delicate (Fig. 1,c) than were those of *bradleyi.* In all specimen of bradleyi examined the predominating spicules along the inner surface of the antennae were stout and darkened by sclerotization or by pigmentation (Fig. 2,c). The darkening was somewhat less pronounced in specimens from Myrtle Beach, S. C., and from Virginia Beach, Va., but the spicules were coarse as compared

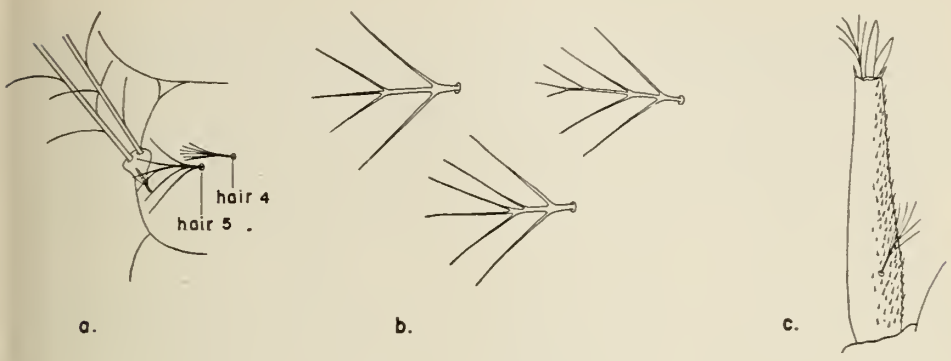

Fig. 1. A. georgianus

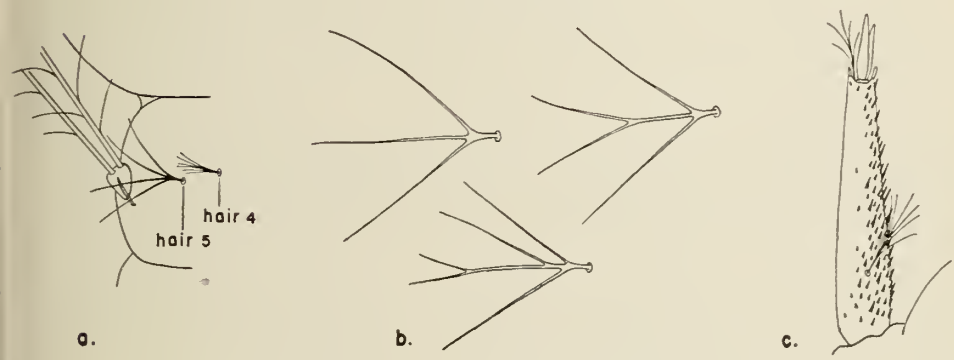

Fig. 2. A. bradleyi

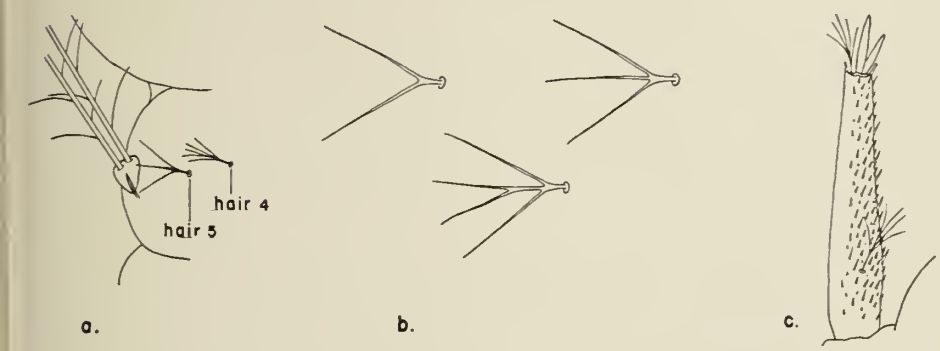

Fig. 3. A. punctipennis

Plate 1.
(*a*) *The left side of abdominal segment I showing typical branching of hair 5 and its size relative with that of hair 4.*
(*b*) *Common variations in the branching of hair 5 of abdominal segment I (drawn to the same scale for the three species).*
(*c*) *Characteristic texture of spinulaton of the antennae.*

with those typical of *georgianus*. With little study of the antennal spicules this character should become a useful diagnostic difference.

The outer occipital hairs reached beyond the bases of the frontal hairs in only one specimen of *georgianus* examined (from Camp Shelby, Mississippi). In five specimens of *bradleyi* these hairs fell slightly short of reaching the frontals but they were longer than those typical of *georgianus* in which species they usually fall far short of reaching the frontals.

The number of branches in hair 5 of abdominal segment I is less distinctive than the arrangement of the branches of this hair. It may be described as a tree-shaped hair not much longer than the branched hair No. 4 above it in *georgianus*, (Fig 1,a) and as a crow-foot shaped hair about twice as long as hair 4 in *bradleyi* (Fig. 2,a). The comparative length with that of hair 4 results both from an increased length of the branches of hair 5 in *bradleyi* and a reduced length of hair 4 in this species.

The inner submedian prothoracic hair, while less consistent than the other characters, in *georgianus* is more likely to be branched and to have more branches, and the branches may arise from the middle or below the middle. In no specimen of *bradleyi* examined did the branches, when present, involve as much as the outer half of this hair.

For distinguishing between larvae of *punctipennis* and *bradleyi* the following supplemental characters may be used:

	punctipennis	*bradleyi*
Hair 5 of abdominal segment I	about equal in size to hair 4, and 3-branched, or may have 4 branches by a splitting of the central branch.	about twice as long as hair 4 and usually with 4 or 5 branches.
Prothoracic hair I	usually with 3 to 5 strong branches arising along central portion.	simple or weakly branched at tip.
Head hair 9	usually with 6 to 10 branches and about equal in size to hair 8.	usually with 3 to 5 branches and longer than hair 8.
Prothoracic hair 1 or the inner submedian prothoracic hair	usually with 3 to 5 branches arising variously along central portion.	simple (about 30 per cent or weakly branched at the tip.

The relative size of hair 5 as compared with that of hair 4 immediately above it on segment I is more characteristic than is the number of branches. The branches of hair 5 are of moderate length and not much longer than hair 4 in *punctipennis* (Fig 3,a) while in *bradleyi* their length is considerably increased (see discussion above and Fig. 2,a).

The inner submedian prothoracic hair is somewhat variable and may appear simple or branched only on its outer end in *punctipennis*, but strong side branches usually present along its center or basal half in this species distinguish it from *bradleyi*.

The outer occipital hair (head hair 9), while involving considerable variation in the number of its branches in both species, its relative size as compared with that of the inner occipital hair (head hair 8), together with the extent of branching, should make this a useful differential character. Furthermore, the failure of head hair 9 to reach beyond the bases of the frontals would distinguish the majority of *punctipennis* from *bradleyi*.

The character of the antennal spicules is less distinctive for *punctipennis* than for *georgianus* in that they are not as fine and delicate in *punctipennis*. In all *punctipennis* examined, however, the spicules were clear and unpigmented (Fig 3,c) as distinguished from the pigmented, coarse spicules present on the antennae of *bradleyi* (Fig. 2,c).

For separating the three species the following key may be found useful:

1. With 3 pairs of functional palmate hairs only (on segments 4 5 and 6); hair 5 of abdominal segment I usually with 5 to 7 branches of variable length arising irregularly and with secondary branching outward from the base (Fig 1,a and b) . . . . . *georgianus*.

   With 5 pairs of functional palmate hairs (on segments 3 to 7 inclusive); hair 5 of abdominal segment I usually with 3 to long branches arising from near the base (Figs. 2a & b and 3,a & b). . . . . 2.

2. Palmates on segments 3 and 7 usually smaller than those on segments 4, 5 and 6, and the leaflets mostly slender and without notches or serrations on the margins; hair 5 of abdominal segment I about twice as long as hair 4 and usually 4 or 5 branched (Fig. 2,a); head hair 9 usually 3 to 5 branched; prothoracic hair I simple or weakly branched at the tip . . . . . *bradleyi*.

   Palmates on segments 3 and 7 about equal in size to the others, and with broad leaflets notched or serrated on the margins; hair 5 of abdominal segment I not distinctly longer than hair 4 and usually 3 branched, (Fig. 3,a), frequently 4 branched, (Fig. 3,b); head hair 9 usually 6 to 10 branched; prothoracic hair I usually with 3 to 5 strong branches arising along the central portion . . . . . *punctipennis*.

Variations in the character differences in the three species, calculated as percentages of the total number of each character present and visible in this series of larvae, are as follows:

	georgianus	*bradleyi*	*punctipennis*
Palmate hairs on segments III and VII:			
Well developed and considered functional	0.0	100.0	100.0
Poorly developed and apparently not functional	100.0	0.0	0.0
Antennal spicules:			
Coarse, spinclike, and pigmented	0.0	100.0	0.0
Fine, slender, and not pigmented	100.0	0.0	100.0
Head hair 9 or outer occipital hair:			
2 - branched	7.1	3.3	0.0
3 - branched	25.9	31.5	1.0
4 - branched	43.4	35.8	1.0
5 - branched	16.4	21.7	12.5
6 - branched	7.1	7.6	29.9
7 - branched	0.0	0.0	30.8
8 - branched	0.0	0.0	16.3
9 - branched	0.0	0.0	6.7
10 - branched	0.0	0.0	1.9
Reaching beyond bases of frontal hairs	2.3	87.0	18.6
Not reaching bases of frontal hairs	97.7	12.0	54.0
Reaching about to bases of frontals but not beyond	0.0	1.0	27.4
Hair 5 of abdominal segment I:			
2 - branched	0.0	0.0	2.1
3 - branched	0.0	8.0	70.0
4 - branched	4.3	40.0	26.9
5 - branched	37.2	46.0	1.0
6 - branched	42.0	4.0	0.0
7 - branched	14.3	2.0	0.0
8 - branched	1.1	0.0	0.0
9 - branched	1.1	0.0	0.0
All branches arising from near base	1.1	74.3	88.7
One or more branches arising farther out	98.9	25.7	11.3
Prothoracic hair 1 or submedian prothoracic hair:			
Simple	4.0	29.7	8.4
2 - branched	5.0	34.0	13.1
3 - branched	28.4	20.4	30.8
4 - branched	29.3	13.6	21.5
5 - branched	25.2	2.3	21.5
6 - branched	8.1	0.0	4.7
Of those branched, the branches arising from:			
The tip only	5.3	51.6	4.2
The apical fourth	17.8	25.7	5.3
The apical third	42.1	22.7	19.0
The apical half	27.4	0.0	16.0
Below the apical half	7.4	0.0	55.5
Tubercles of inner clypeal hairs separated by:			
Less than half the tubercle width or touching	14.8	0.0	50.0
Approximately half a tubercle width	51.8	9.6	45.0
Slightly less than a full tubercle width	24.2	40.4	3.3
Fully the width of one tubercle	9.2	27.0	1.7
Distinctly more than the width of one tubercle	0.0	23.0	0.0
Posterior clypeal hairs:			
Simple	43.3	89.6	28.5
2 - branched	52.2	9.2	50.9
3 - branched	1.1	0.0	15.2
4 - branched	3.3	1.2	5.2
With branches arising from			
The apical half	31.0	4.6	51.0
The basal half	26.0	5.8	20.5
Antepalmate hair or hair No. 2:			
On abdominal segment IV:			
Simple	4.0	49.0	9.1

2 - branched	9.0	33.6	65.4
3 - branched	58.7	17.4	23.7
4 - branched	14.2	0.0	1.8
5 - branched	11.1	0.0	0.0
6 - branched	2.0	0.0	0.0
On abdominal segment V:			
Simple	0.0	47.9	15.5
2 - branched	2.2	34.1	60.0
3 - branched	68.1	15.9	24.5
4 - branched	19.8	2.1	0.0
5 - branched	8.8	0.0	0.0
6 - branched	1.1	0.0	0.0
Lateral hairs on abdominal segment IV:			
Branches arising from the same point	42.0	86.0	100.0
With irregular branching	58.0	14.0	0.0
Lateral hairs on abdominal segment V:			
Branches arising from the same point	77.0	94.5	100.0
With irregular branching	23.0	5.5	0.0
Leaflets of palmate hairs on segment III:			
Mostly with notched or serrated margins		31.1	100.0
Mostly with smooth margins		68.9	0.0
Leaflets of palmates on segment VII:			
Mostly with notched or serrated margins		7.9	100.0
Mostly with smooth margins		92.1	0.0

This tabulation shows agreement with the difference as noted by King and others with the following exceptions:

(1) The branching of the posterior clypeal hairs may involve more than the apical half of the hair in both *georgianus* and *bradleyi*. It is also noted that this hair may rarely have as many as four branches in both these species as well as in *punctipennis*.

(2) The antepalmate hairs on segments 4 and 5 may be simple or two-branched in *georgianus* and they are frequently triple and may rarely be four-branched in both *bradleyi* and *punctipennis*.

(3) The leaflets of the palmates on segment 3 of *bradleyi* are often notched or serrated on the margins, causing confusion of this species with *punctipennis*.

As a characteristic of *georgianus* it is worthy of note that in this species there is a tendency toward more branching of all the hairs studied, both in number of branches and in the extent of sub-branching of branches outwardly.

No distinguishing differences were found in the antennal hairs nor in the tergal plates. The pecten and respiratory apparatus of the eighth abdominal segment were not studied in this series.

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ANOPHELES AQUASALIS vs. ANOPHELES TARSIMACULATUS AS THE NAME FOR THE BRACKISH WATER ANOPHELINE OF CENTRAL AND SOUTH AMERICA AND THE CARIBBEAN ISLANDS*

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The controversy pertaining to the validity of the name *tarsimaculatus* has assumed prominence with the recognition that the mosquitoes so designated constitute several valid species. A brief resume of the origin and early application of the name *tarsimaculatus* will facilitate a comprehension of the problem involved. The mosquitoes to be considered are those of the subgenus *Nyssorhynchus* which have a black ring on the fifth hind tarsal segment. The first mosquito of this group to be described was *Anopheles albimanus* Wiedemann 1821. Theobald (1901) later described the same mosquito as *Anopheles argyritarsis* variety *albipes* thinking that it was a variety of *Anopheles argyritarsis* Robineau-Desvoidy 1827. Theobald (1903) raised his *albipes* to specific rank and placed it in the genus *Cellia*. Goeldi (1905) published a collection of four papers under the title "Os Mosquitos no Pará. Reuniao de quatro trabalhos sobre os mosquitos indigenas, principalmente as especies que molestam o homen" in which he followed the nomenclature of Theobald. In the first of these papers Goeldi (1902) also (1905) states that the Anophelines of principal interest in Pará are *Anopheles argyritarsis* and *Anopheles argyritarsis* var. *albipes*. In a third paper of the same series Goeldi (1905) designated the Anophelines of Pará as *Cellia argyritarisis* var. *albitarisis* and *Cellia argyritarsis* var. *albipes*. Since *albitarsis* and *albipes* both mean "white feet," and since the mosquito called *albipes* in reality had a black band on the fifth hind tarsi Goeldi proposed to change this latter to *tarsimaculatus*. No description, however, was made of the mosquito which Goeldi thus designated as *tarsimaculatus* except for an illustration of the posterior tarsi and photographs of eggs at very low magnification, and of first stage larva. Other photographs of eggs at much higher magnification are also shown, probably from the same oviposition but Goeldi does not make a specific statement to this effect. The photographs are either badly reproduced or

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were retouched. It is obvious that the frill at each end of the egg in Fig. 123 of Plate O does not protrude over the tips, while the frills of those in Fig. 131 extend considerably over the tips and can be seen from the ventral side of the egg (dorsal side of the larva). The eggs depicted in Fig. 131 are similar to the eggs of *Anopheles goeldii* Rozeboom and Gabaldon (1941). The Fig. 123 resembles the egg of *Anopheles aquasalis* Curry (1932) while that in Fig. 133 is unlike any known anopheline egg in Brazil. The tarsal segments shown in Fig. 10 b, Plate II, can be associated with several species. In the areas studied by Goeldi the mosquitoes with tarsi similar to this drawing are *Anopheles goeldii, Anopheles aquasalis* and *Anopheles triannulatus*. Goeldi states in his first paper that the Anophelines which he was describing as var. *albipes* (later called *tarsimaculatus*) were from Macapá where collectors of the museum had worked for three years. Recent investigations by the authors to determine what species now exist in Macapá reveal the prevalence of large numbers of *Anopheles goeldii* and *Anopheles triannulatus* but no *Anopheles aquasalis*. In Belem, another region studied by Goeldi, *Anopheles aquasalis* is now prevalent, but *Anopheles goeldii* is not found. In addition to these three species which have hind tarsi corresponding to Goeldi's diagram, there exist in Pará *Anopheles oswaldoi,* and *Anopheles konderi* which have a narrower ring on the second hind tarsi but due to the presence of a black ring on the fifth hind tarsi would certainly have been identified by Goeldi as *albipes* (or *tarsimaculatus*). From these observations it may be deducted that Goeldi not only collected but designated several species of Anophelines as *albipes* (*tarsimaculatus*).

Dyar and Knab (1906) realized that the anophelines of Pará were not *Anopheles albipes* Theobald 1903 as assumed by Goeldi, but constitute a different species for which they used Goeldi's new name *tarsimaculatus*. Theobald (1907), however, placed both *albipes* and *tarsimaculatus* in synonymy with *albimanus* because he had discovered that his original *albipes* was the same as *albimanus* and he thought that the specimens from Pará were identical with them. Peryassu (1922) recognized that the mosquitoes with a narrow black ring on the second hind tarsi formed an entity distinct from those that had a large ring. For the former he proposed the name *Anopheles oswaldoi*. Root (1926) did not consider *oswaldoi* a valid species and continued to use the name *tarsimaculatus,* not only for *oswaldoi* but for other mospuitoes subsequently shown to constitute several species. Rozeboom and Gabaldon (1941), reported finding four distinct species among the specimens labelled *tarsimaculatus* in the collection of the late Dr. F. M. Root. Two additional species have been found among specimens mounted on four slides that were given to one of the authors by Dr. Root. Town-

send (1933) called attention to the fact that the name *tarsimaculatus* was illegally proposed and should not be used. For the mosquito so designated by Dyar and Knab he proposed to revalidate *Anopheles gorgasi* Dyar and Knab, 1907, which, however, Rozeboom and Gabaldon (1941) consider to be a synonym of *albimanus*. Curry (1932) separated two varieties of *tarsimaculatus* on the basis of biological and morphological characteristics. These he called *Anopheles tarsimaculatus* var. *aquasalis* and *Anopheles tarsimaculatus* var. *aquacoelestis*. The first has been raised to specific rank by Rozeboom and Gabaldon and the latter placed in synonymy with *oswaldoi*. It is of interest to note that this is the first time the name *tarsimaculatus* had been associated with descriptions which served to characterize accurately the mosquitoes designated.

Several workers in Brazil also recognized that the mosquitoes commonly referred to as *Anopheles tarsimaculatus* constituted several varieties or species. Galvao and Lane (1938) proposed the revalidation of *Anopheles oswaldoi* Peryassu 1922 and described two varities of the species as *Anopheles oswaldoi oswaldoi* and *Anopheles oswaldoi noroestensis*. Galvao (1940) continued the discussion of these varities. Unfortunately the descriptions in both of these papers were not based on homogeneous material. In the first paper Galvao and Lane created the variety *metcalfi* for the mosquitoes that oviposited the peculiar type of egg described by Root (1926). It is now generally recognized that Root's description was erroneous. Meanwhile Coutinho (1942) has published data obviously pertaining to Curry's *aquasalis* under the name of *Anopheles oswaldoi* var. *metcalfi*. Unti (1940) described an additional variety as *Anopheles oswaldoi* var. *ayrozai*. His descriptions of the eggs, larvae, pupae and adult were made on material reared in the laboratory from females captured on animal bait. Galvao and Damasceno (1942) raised *noroestensis* to the rank of species and at the same time verified that Unti's *ayrozai* is a synonym of *noroestensis*. Komp (1942) described *Anopheles clarki* which is also a synonym of this species. Our dissections on numerous male genitalia of mosquitoes collected in northeast Brazil confirm the synonymy of *clarki* with *noroestensis*.

Galvao and Lane (1938) considered Goeldi's name *Anopheles tarsimaculatus* valid but realized that no known mosquito could be recognized by this name on the basis of recorded descriptions. In an attempt to clarify the issue they described an Anopheline from the interior of the Amazon Valley and designated it as *Anopheles tarsimaculatus* Goeldi, 1905. To support their claim for the validity of this name they quote article 21 of the International Rules of Zoological Nomenclature, as follows:

"Article 21.—The author of a scientific name is that person who first publishes the name in connection with an indication, a definition, or a description, unless it is clear from the contents of the publication that some other person is responsible for said name and its indication, definition, or description."

Rozeboom and Gabaldon (1941) do not agree that article 21 is applicable, and to disprove the validity of the name *tarsimaculatus* as applied by Goeldi to the variety *albipes* they quote article 32 of the International Rules of Zoological Nomeclature, as follows:

"Article 32.—A generic or a specific name, once published, cannot be rejected, even by its author, because of innapropriateness. Examples: Names like *Polydon, Apus, albus,* etc.,. when once published, are not to be rejected because of a claim that they indicate characters contradictory to those possessed by the animals in question."

Along with a review of the *tarsimaculatus* complex Rozeboom and Gabaldon (1941) described an Anopheline from the Amazon Valley as *Anopheles goeldii.* This mosquito incidentally is undoubtedly the same species described by Galvao and Lane (1938) as the type material for *Anopheles tarsimaculatus* and by Townsend (1933) as the revalidated *Anopheles gorgasi.* Komp (1941) in a discussion concerning the validity of *tarsimaculatus* presents an argument similar to that of Rozeboom and Gabaldon, and states that no one had taken into consideration the possibility that the species from Belem, Pará might be different from anything so far described from the area. In order to investigate this possibility he made a visit to Belem for a few days in April 1941 to obtain material of Goeldi's species. He collected specimens which he considered different from *oswaldoi, nunez-tovari, rangeli* and *aquasalis.* He described the species and for it proposed the name *Anopheles emilianus.* Galvao, Damasceno, and Marques (1942) who were working in Belem at the time of Komp's visit and assisted Komp in collecting his material, still retain the name *tarsimaculatus* for this species in Belem. These observers after an extensive study of the mosquitoes of Belem report that *A. tarsimaculatus* (*A. emilianus* Komp 1941) is chiefly found in breeding places with relatively high chloride concentration, on the low lands which are invaded by the tide. It is unfortunate that these investigators used the name *tarsimaculatus* in their excellent study of the biology of this brackish water mosquito as they realized it to be different from the mosquito Galvao and Lane 1938, had described and designated as*Anopheles tarsimaculatus* from the Rivers Parauarí, Maúes, and Manacapurú, and which is now recognized as *Anopheles goeldi*

with an entirely different biology. Ramos (1942) described *Anopheles oswaldoi* var. *guarujaensis* which is evidently *Anopheles aquasalis* and which was placed in synonymy with *tarsimaculatus* by Galvao and Damsceno (1942).

During the past four years the authors of this paper have worked constantly with this coastal species variously known as *tarsimaculatus, guarujaensis, metcalfi, emilianus* and *aquasalis. It has been* collected along the coastal region from Rio de Janeiro to the French Guiana border, always in areas influenced by tidal sea water. Extensive searches for this mosquito in the Amazon region have revealed that the species does not exist in the interior from Belem. The biology of *aquasalis* in Brazil will be discussed in a separate paper in the near future. It will suffice to state here that the chloride content of many breeding places has been determined and the type of water collections preferred by *aquasalis* noted. Although the species can and does mature in fresh water it is usually found in brackish water. The species is particularly resistant to salt water. Under laboratory conditions larvae can mature in sea water. In one experiment when sea water was permitted to evaporate until it reached a concentration of 7.4 percent sodium chloride, larvae continued to grow and pupated.

In making the distinction between *Anopheles emilianus* and *Anopheles aquasalis* Komp states that "the male terminalia are not particularly distinctive although the mesosome of *aquasalis* has a shorter and broader tip than that of *emilianus,* and that the hairs fringing the free margins of the basal lobules of the fused claspette lobes are shorter than in *emilianus.* These differences are variable and are rather indefinite. The species are most easily separated in the egg stage and it is by this criterion that the presence of *aquasalis* and *emilianus* in any locality should be judged."

More than 7000 ovipositions from isolated females of this species have been obtained and studied. Considerable variation in the type of eggs oviposited by this mosquito has been observed. In Ceará where the climate is dryer the eggs appear closed, with floats more closely approximated. In Belem the eggs show a greater tendency to appear open with the floats separated. Even among eggs of the same oviposition, however, both open and closed types can be observed. Variations in the male genitalia as great as the differences described by Komp between *emilianus* and *aquasalis* may also be found among mosquitoes from the same oviposition.

After making extensive studies on this mosquito in Ceará and in Belem the authors fail to find any characteristics either biological or morphological that separate the brackish water breeding Anopheline of Brazil from *Anopheles aquasalis* Curry 1932 or to find

any other mosquito that conforms to the description given for *Anopheles emilianus*. Therefore *Anopheles emilianus* is considered to be synonymous with *Anopheles aquasalis*.

The following mosquitoes of the subgenus *Nyssorhynchus* with a black ring on the fifth hind tarsi have been described. With the exception of *albimanus* all the valid species have been found in the northeast and Amazon regions of Brazil.

Anopheles albimanus Wiedermann, 1821
= *argyritarsis* var. *albipes* Theobald, 1901
= *albipes* Theobald, 1903
= *tarsimaculata* Goeldi, 1905
= *gorgasi* Dyar and Knab, 1907
Anopheles aquasalis Curry, 1932.
= *tarsimaculatus var. aquasalis* Curry, 1932
= *tarsimaculatus Galvao, Damasceno and Marues,* 1942 (not Goeldi, 1905, nor Galvao and Lane, 1938)
= *emilianus* Komp, 1941
= *oswaldoi var. guarujaensis* Ramos, 1942
= *oswaldoi* var. *metcalfi Coutinho,* 1942 (not Galvao and Lane 1938).
Anopheles benarrochi Gabaldon, Cova-Garcia and Lopez, 1941
Anopheles dunhami Causey, in press.
Anopheles galvaoi Causey, Dean and Deane, 1944
Anopheles goeldii Rozeboom and Gabaldon, 1941
= tarsimaculatus Galvao and Lane, 1938 (not Goeldi, 1905)
= *gorgasi* Townsend, 1933 (not Dyar and Knab, 1907)
Anopheles konderi Galvao and Damasceno, 1942
Anopheles noroestensis Galvao and lane, 1938
= *oswaldoi ayrozai* Unti, 1940
= *clarki* Komp, 1942
Anopheles oswaldoi Peryassu, 1922
= *tarsimaculatus var. aquacoelestis* Curry, 1932
Anopheles rangeli Gabaldon, 1940
Anopheles triannulatus Neiva and Pinto, 1922
Anopheles strodei Root, 1926
Anopheles thriannuatus Neiva and Pinto, 1922
= *Anopheles bachmani* Petrocchi, 1925
Anopheles oswaldoi var. metcalfi Galvao and Lane, 1938 (*nomen nudum?*)

Summary

The validity of the name *tarsimaculatus* as used by Goeldi for the Anophelines of the sub-genus *Nyssorhynchus* with black ring on the fifth hind tarsi, is discussed. It is pointed out that at least three species of the *Nyssorhynchus* group corresponding to Goeldi's meagre descriptions are present in the areas from which he collected his material. It is evident that the name was used by him, not for one but for several species. The mosquitoes in Belem designated as *Anopheles tarsimaculatus* by Galvao, Damasceno and Marques (1942), and as *Anopheles emilianus* by Komp (1941) are indistinguishable in morphological and physiological characteristics from *Anopheles aquasalis* Curry 1932. The authors are of the opinion that since *tarsimaculatus* was illegally proposed as an emendation of a name considered inappropriate, and was applied to mosquitoes subsequently differentiated as several distinct species, the name is not available for any Anopheline. *Anopheles emilianus* Komp 1941 is considered to be a snyonym of *Anopheles aquasalis* Curry, 1932.

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OBSERVATIONS ON SEASONAL OCCURRENCE AND ABUNDANCE OF *ANOPHELES QUADRIMACULATUS* SAY*

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During the course of the entomological survey and surveillance work of the Malaria Control in War Areas program which the Public Health Service has prosecuted during the past three years in cooperation with various state health departments, considerable data have been accumulated on the seasonal and local distribution and abundance of adult *Anopheles quadrimaculatus* throughout the Southeastern United States. This paper presents by means of a series of charts certain generalizations which may be made from these data on the length of the active *Anopheles quadrimaculatus* season in the several groups of isotherms of the region and the abundance of these mosquitoes as measured by index station observations. In addition, other charts are presented showing conditions in certain specific localities. Semi-logarithmic graph paper has been used in charting the data. The term "significant" densities or populations as used in this paper indicates the finding of 10 or more *quadrimaculatus* in any resting place in a locality. On the Malaria Control in War Areas program this criterion has been used to determine those localities in endemic malaria areas where control work is necessary and also as a guide in evaluating progress when control is prosecuted. The records used in making these studies were obtained chiefly from index stations located adjacent to but beyond the limits of protected areas and thus represent densities little affected by control operations.

Figure 1 shows the zones into which the United States may be divided by isotherms drawn at 5°F intervals[1]. Each of these zones, representing an area in which the average annual temperature varies only within 5 degrees, has been used as a unit in preparing summaries of data.

The maximum numbers of adult *A. quadrimaculatus* observed in natural resting place index stations such as barns, stables, privies, etc., in 5 different isothermal zones of the Southeastern United States during the three year period 1942-44 are shown by the graphs of Figures 2 through 6. Isothermal zone 70° to 75° F includes the

(1) Based on data included in Climate and Man, U. S. Department of Agriculture, Washington, D. C., 1941.

*This paper was presented at the annual meetings of the National Malaria Society in St. Louis, Missouri, 14 November 1944.

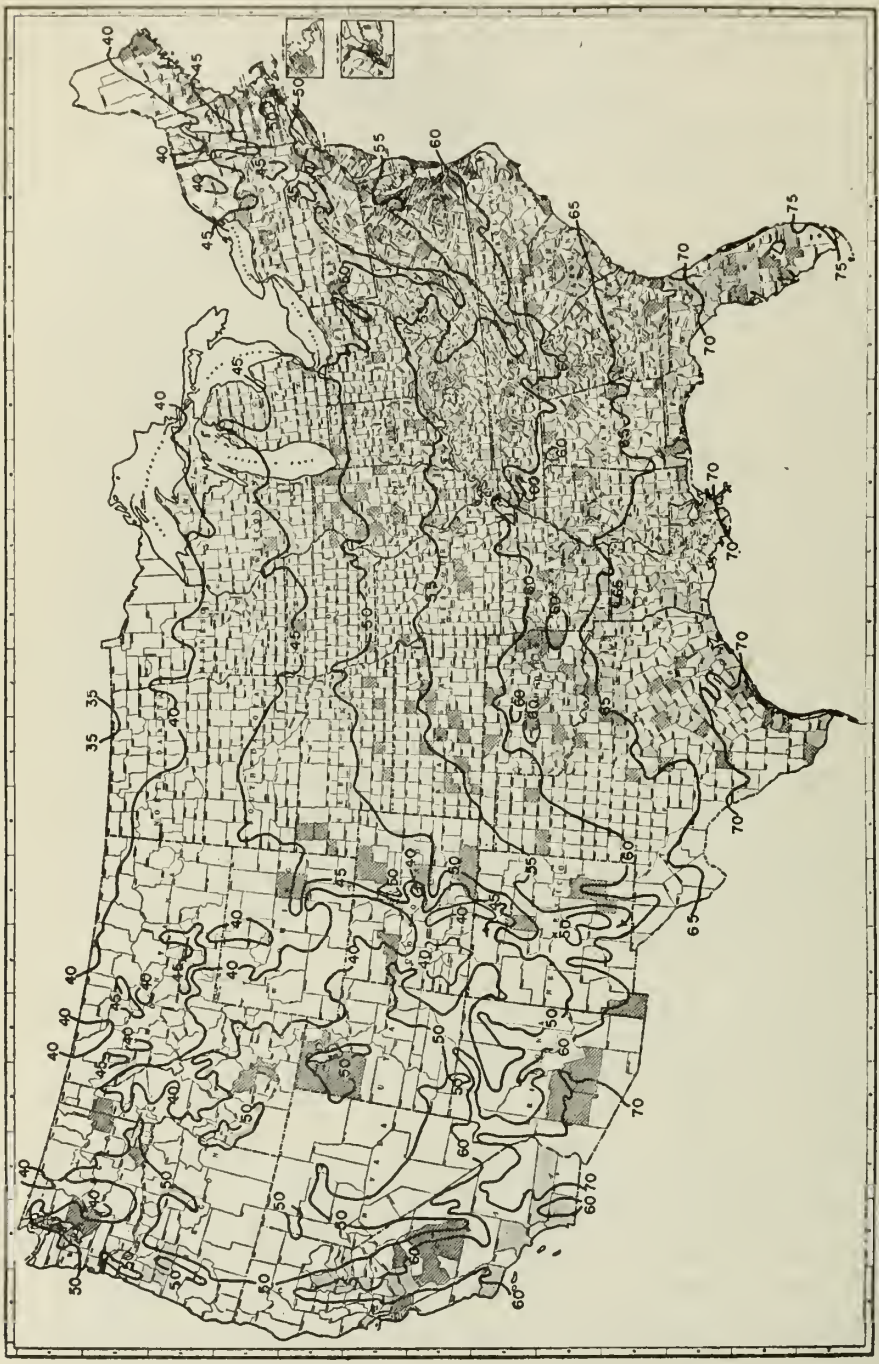

Fig 1. *Map of United States with isotherms drawn at 5°F intervals. Background shading indicates counties where entomological surveillance or inspection and control projects are operated by Malaria Control in War Areas.*

most southerly region of the United States except the southern tip of Florida and covers most of peninsular Florida, southern Louisiana and Texas. In this isothermal zone, significant densities of *A. quadrimaculatus* occur throughout the year (Figure 2). Above 100 *quadrimaculatus* in adult resting stations were found continuously during the 8 warmer months in this zone and the maximum *quadrimaculatus* population recorded for a single index station was 2500. Peaks of abundance were recorded in June and September.

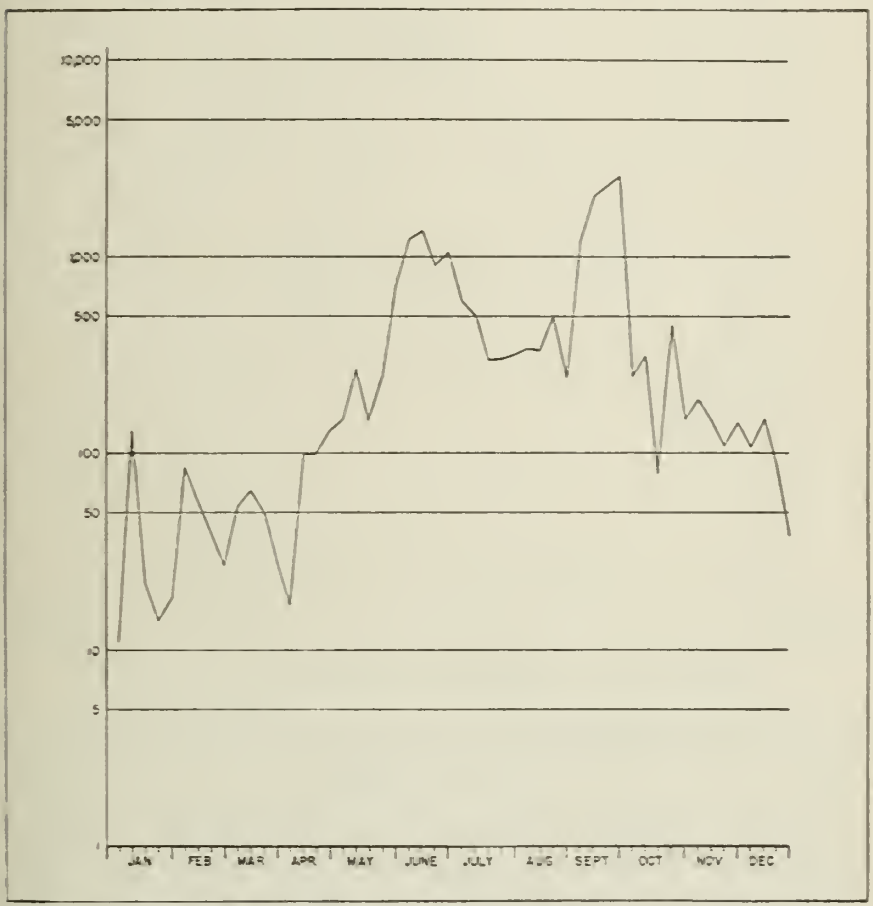

Fig 2. Maximum adult densities of A. quadrimaculatus in Isothermal Zone 70°—75°, 1942-1944.

Between isotherms 65° and 70° are included part of east Texas; nearly all of Louisiana; the southern parts of Mississippi, Alabama, South Carolina and Georgia and northern Florida. Conditions here (Figure 3) are similar to those in the preceding zone; significant *quadrimaculatus* densities occurred throughout the year and natural resting place counts above 100 were encountered during the 8 con-

secutive warmer months. A maximum index station population of 4000 *quadrimaculatus* was reported from this area and counts of more than 1000 were found from the last of May through August.

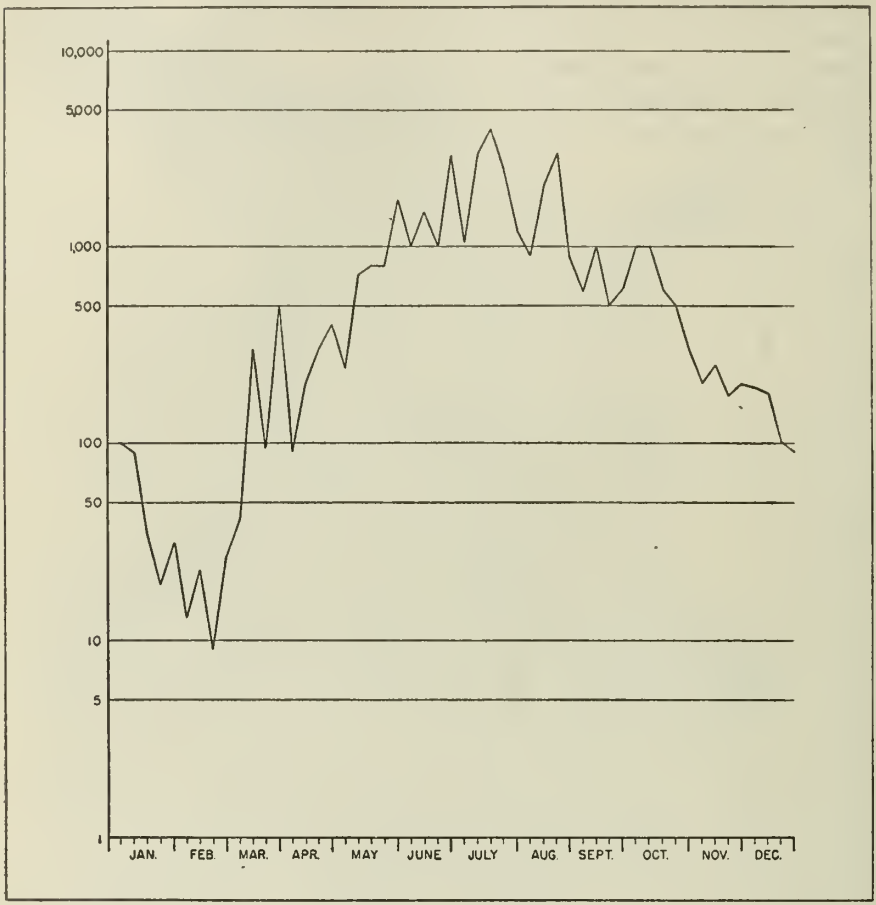

Fig. 3. Maximum adult densities of A. quadrimaculatus in Isothermal Zone 65°–70°F, 1942-1944.

Between isotherms 60° and 65° are included parts of northern Texas, nearly all of Oklahoma and Arkansas, southwestern Tennessee, the northern part of Mississippi, Alabama and Georgia, and much of South Carolina and North Carolina. There is a break in the continuity of year 'round significant densities of *Anopheles quadrimaculatus* in this zone (Figure 4). Ten or more adults were observed in individual resting places for a period of only 8 months and of above 100 for less than 6 months. A period of low or zero densities occurred from the middle of December until the first of April with no adults being found between February 13 and March 20.

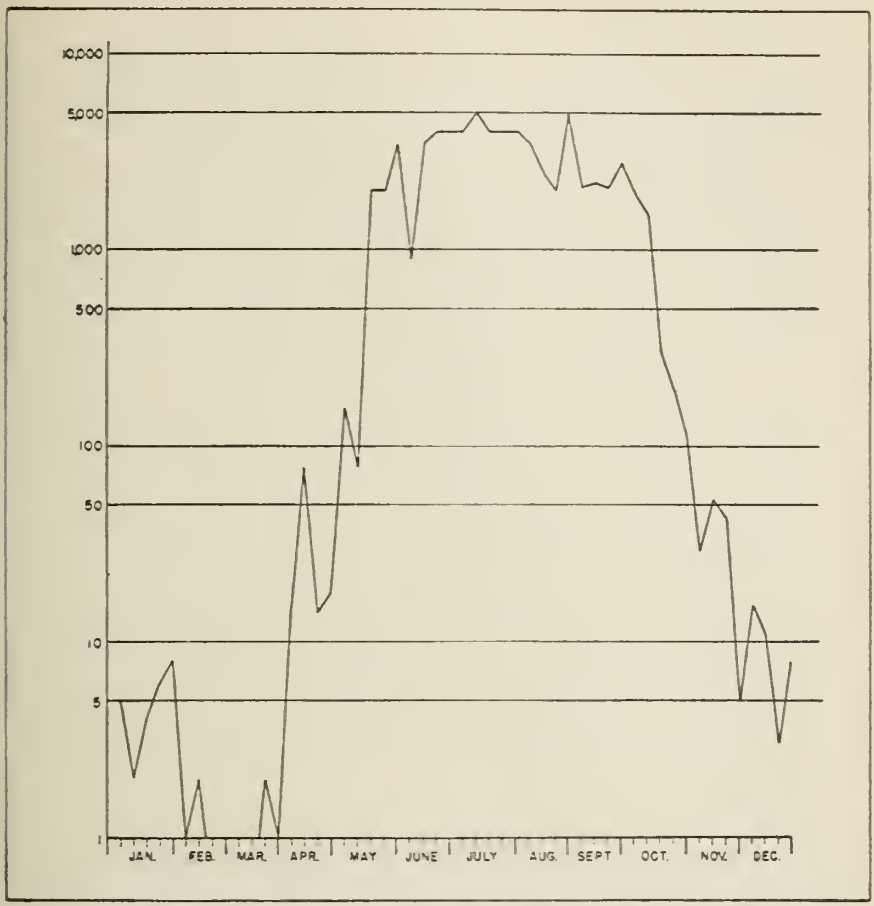

Fig. 4. Maximum adult densities of A. quadrimaculatus in Isothermal Zone 60°–65°F, 1942-1944.

Between isotherms 55° and 60° are included the Texas panhandle, northern Oklahoma, southwestern Kansas, the southern parts of Missouri, Illinois and Indiana, the northern part of North Carolina, and most of Tennessee, Kentucky and Virginia. Index station counts of 10 or more *quadrimaculatus* occurred in this zone for a period of approximately 5½ months and of 100 or more for approximately 4½ months (Figure 5).

Between isotherms 50° and 55° are included northern Kansas, southern Nebraska, northern Missouri, central Illinois, Indiana and Ohio, and parts of Maryland, New Jersey, New York and Delaware. Here the duration of the *A. quadrimaculatus* season (Figure 6) was not greatly different from that occurring between isotherms 55° and 60°. Ten or more *quadrimaculatus* were found in individual resting places for a period of slightly less than 5 months and of 100 or

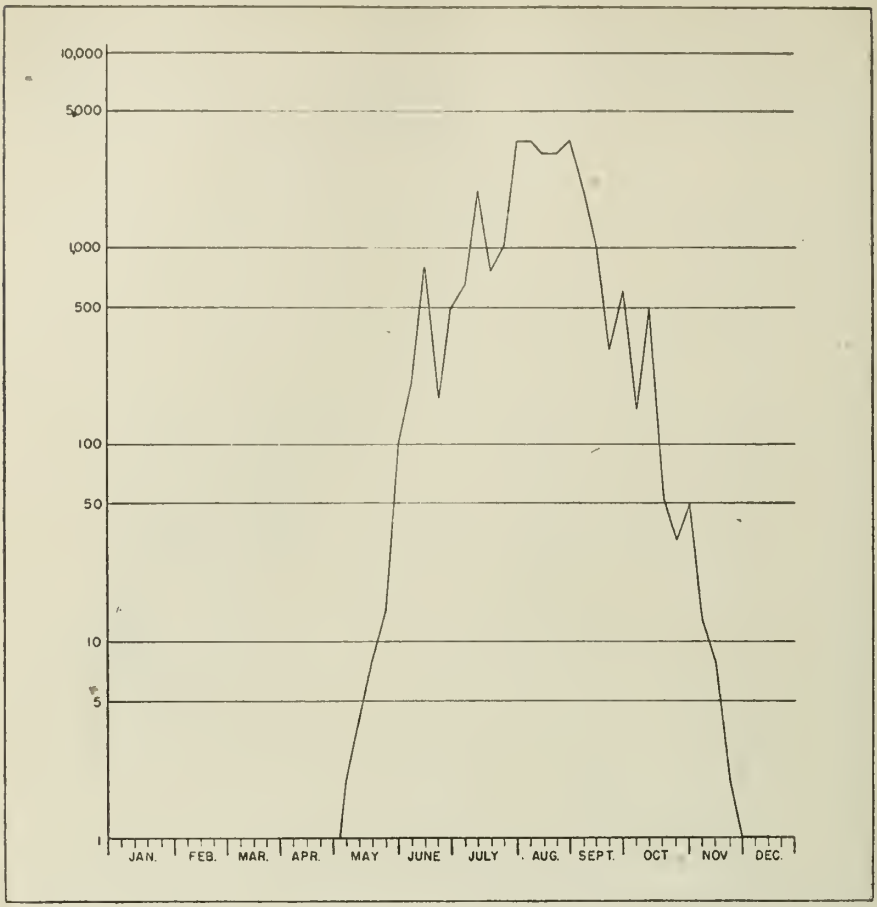

Fig. 5. Maximum adult densities of A. quadrimaculatus in Isothermal Zone 55°–60°F., 1942-1944.

more for 4 months. A maximum index station population of over 9000 *quadrimaculatus* was reported in this zone. Our entomological records for areas north of isotherm 50° are insufficient to allow further comparisons.

It is interesting to note that in the more northerly zones high densities of *quadrimaculatus* occur at the very beginning of the active season and abruplty disappear at the end of the season. Also the abundance of this mosquito, as indicated by index station populations, may be greater in the northern latitudes than farther south where the active season is of much longer duration.

Figure 7 shows diagrammatically the length of the season of significant *A. quadrimaculatus* populations based on records for the past three seasons in the different isothermal groups under consideration. The present endemic malarious areas of eastern United States are included between isotherms 60° and 75°. Between iso-

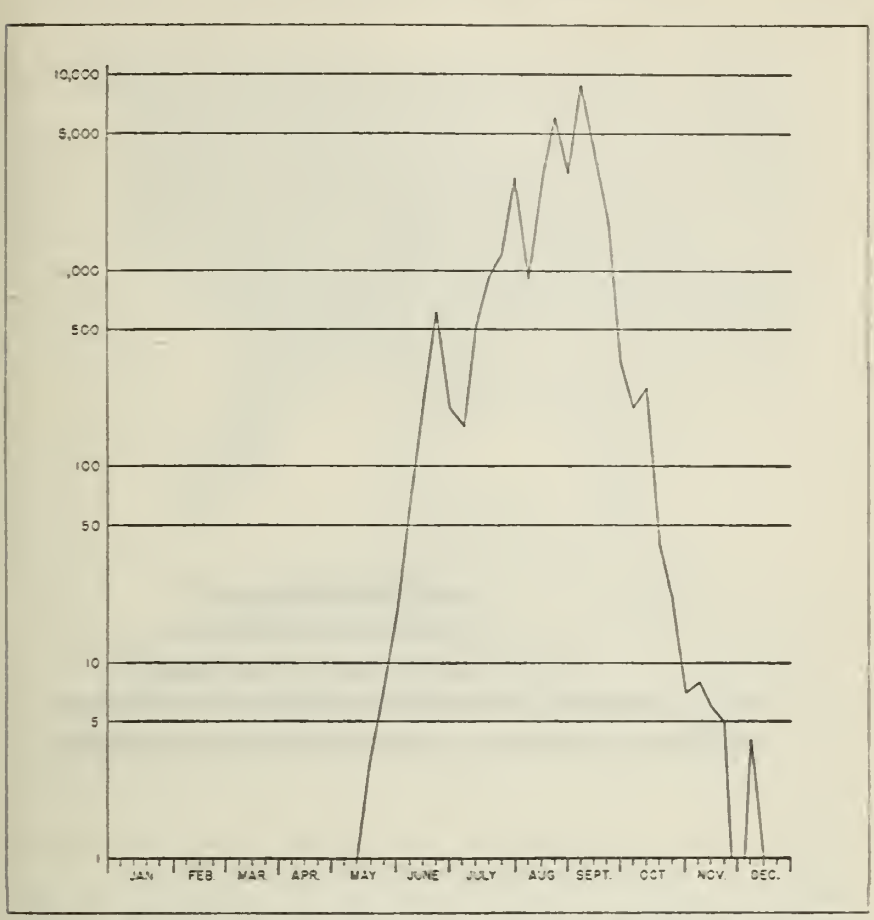

Fig. 6. Maximum adult densities of A. quadrimaculatus in Isothermal Zone 50°—55°F, 1942-1944.

therms 65° and 75° significant numbers of *quadrimaculatus* were found throughout the year. Between 60° and 65° significant numbers did not occur from late November through March, but a few active specimens were found at all times during the winter except for a short period during late February and early March. This was not the case in the two most northerly zones included in this study. In these the *quadrimaculatus* populations either died or entered hiberation in the fall and did not again become active until May.

In Figure 8 the per cent of localities under observation in isothermal zone 65° to 70° in which significant densities of *quadrimaculatus* occurred is shown for each week during the years 1943 and 1944 . During the first three months of 1943 practically none of the localities had important densities of this mosquito. Beginning in April a gradual increase occurred which reached the approximate peak for the season during the first week in July. In 1944 signi-

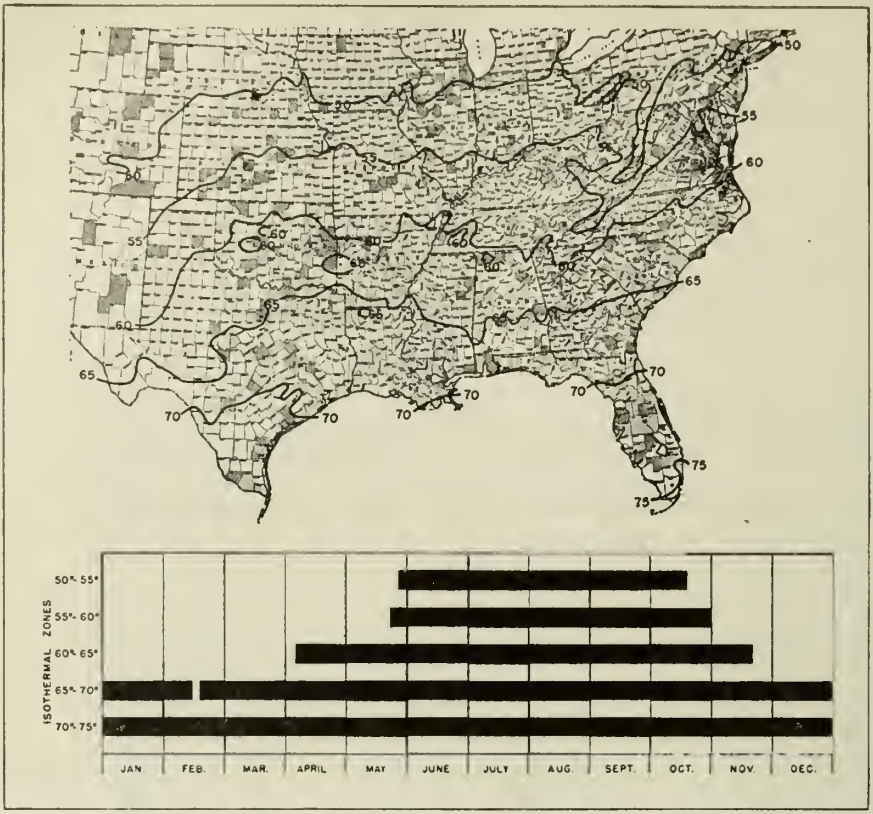

Fig. 7. Bar graphs showing the length of the signficant A quadrimaculatus season in each of the Isothermal Zones shown on the map.

ficant densities persisted during the winter in a considerable number of localities. The seasonal buildup began in March, about a month earlier than in 1943, reaching a peak of 53 per cent during the middle of June. This is to be compared with the maximum of 43 per cent reached during July of the preceding year. In 1944 not only was the *quadrimaculatus* season of greater length but also the percentage of localities having important densities was greater.

The preceding graphs have shown the length of the season of adult *quadrimaculatus* activity and maximum populations as indicated by the numbers occurring in resting places in war areas in the 5 isothermal zones of the southeastern United States. The populations in any one locality depend on numerous local factors associated with *quadrimaculatus* production in that locality. The graphs of Figures 9 through 12 show density conditions which occured during 1943 in four individual localities, three of which are in isotherm group 60° to 65° and one in 65° to 70°. At Newport, Arkansas (Figure 9) the *quadrimaculatus* season was short compared with the composite pattern of the isotherm group (Figure 4). A peak of a-

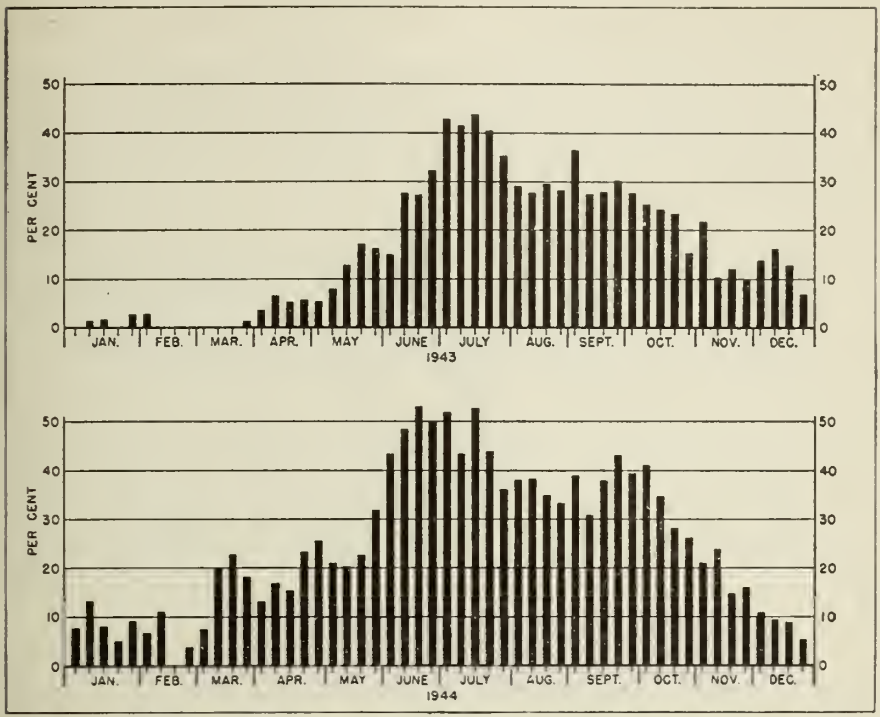

Fig. 8. Bar graphs showing the per cent of zones under weekly observation in Isothermal Zone 65°-70°F, where significant densities of A. quadrimaculatus occured during 1943 and 1944.

bundance occurred in the last week of June followed by a secondary rise in abundance in late September and early October. At New Bern, N. C. (Figure 10) abrupt variations in populations are indicated with the peak of abundance coming in early September. At Sumter, South Carolina (Figure 11) the general pattern of length of season for the isotherm group occurred but with lower populations. At Centerville, Mississippi (Figure 12) a relatively short season of important densities occurred although a long season of high densities is the general pattern in its isotherm group.

From the foregoing it will be seen that conditions likely to occur in any individual locality during the active *quadrimaculatus* season should not be judged by surveys of short duration. This was strikingly illustrated by the experience at Centerville, Mississippi (Figure 12). A military establishment was activated at Centerville during the summer of 1942 and the area was kept under entomological surveillance throughout the remainder of that year. As is shown in the figure, only negligible densities of *quadrimaculatus* were encountered. Some minor drainage and clearing of actual and potential mosquito breeding places was accomplished during the following winter and the area was considered to be satisfactorily cared for.

Fig 9. Maximum A. quadrimaculatus densities at Newport, Arkansas Isothermal Zones 60°-65°F, during 1943.

This did not prove to be the case, however, as high adult *quadrimaculatus* populations occurred during the following June and July which necessitated further control work. Such instances emphasize the importance of entomological surveillance activities on malaria control programs. Localities which may not have significant densities of malaria carrying mosquitoes when first surveyed should be considered in relation to the general pattern of *quadrimaculatus* occurrence in their isotherm zone and frequent inspections made during the active mosquito season.

Summary

Data are presented to show the length of the active *Anopheles quadrimaculatus* season in various isotherms of the United States and the abundance of this mosquito as determined by natural resting place observations.

Examples are given to show how adult densities and the length of the *quadrimaculatus* season in any specific locality may vary from the composite pattern for the isotherm.

The importance of entomological surveillance is discussed.

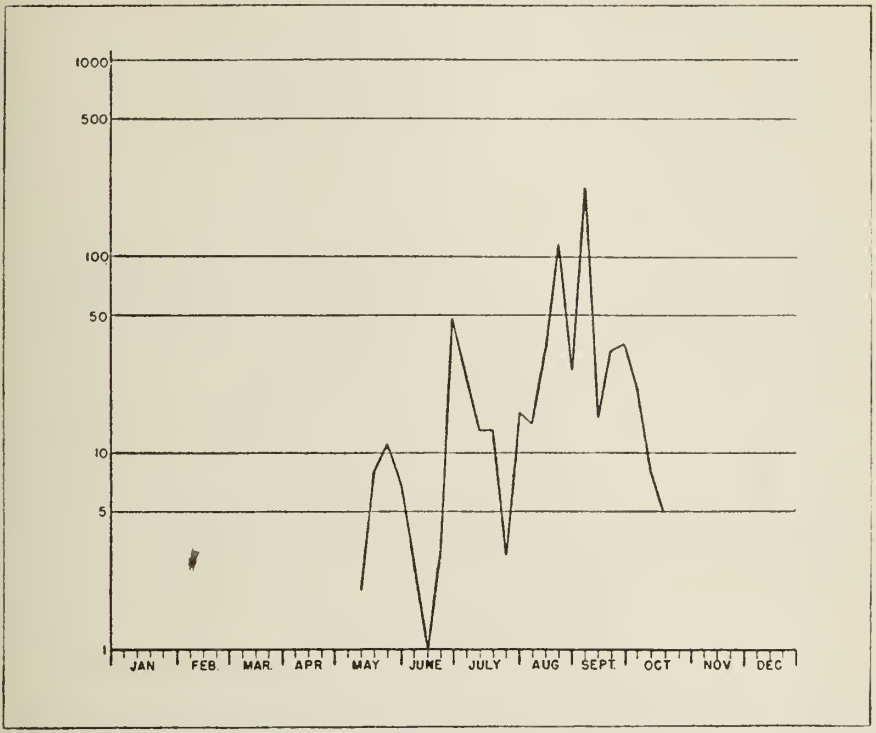

Fig. 10 Maximum adult A. quadrimaculatus densities at New Bern, North Carolina, Isothermal Zone 60°-65°F, during 1943.

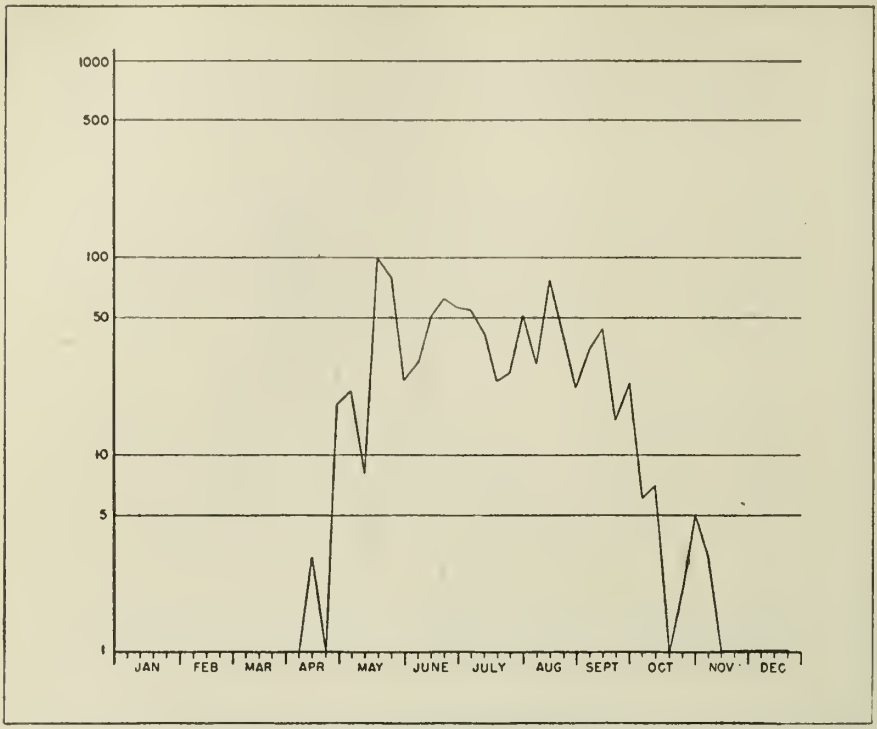

Fig 11. *Maximum adult A. quadrimaculatus densities at Sumter, South Carolina, Isothermal Zone* 60°-65°F *during* 1943.

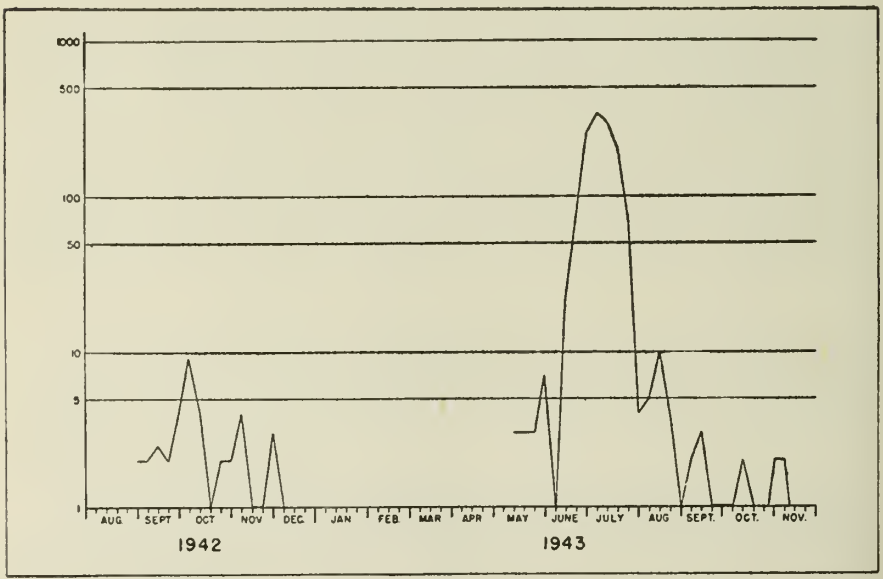

Fig. 12. *Maximum adult A. quadrimaculatus densities at Centerville, Mississippi Isothermal Zone* 65°-70°F *during* 1942 *and* 1943.

DETAILED OBSERVATIONS ON THE LIFE HISTORY OF *ANOPHELES QUADRIMACULATUS**

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(Received for Publication 14 November 1944)

Introduction

Since the spring of 1937, an insectary colony of *Anopheles quadrimaculatus* has been maintained in the malaria control laboratories of the Health and Safety Department of the Tennessee Valley Authority at Wilson Dam, Alabama. Basic techniques used in running this insectary have been described by Crowell (1940). During the past few years, advantage has been taken of the opportunity to make detailed observations on the life history of *A. quadrimaculatus* in connection with the routine management of the insectary. The information obtained has included data on the life cycle of *A. quadrimaculatus* and the egg-laying and feeding habits of the adult mosquitoes. The purpose of the present paper is to present a brief summary of this information.

Rates of Development

Crowell (1940) has briefly summarized the rates of development of *A. quadrimaculatus* under insectary conditions and Hurlbut (1943) has published the results of rather detailed studies on the rate of growth of *Anopheles quadrimaculatus* in relation to temperatures in the Tennessee Valley. Similar data from the present studies are given here for comparison with observations of these workers. During the course of these observations the air temperatures in the insectary have been maintained between 76 and 80° F., and the water temperatures have ranged between 72 and 76° F., generally averaging about 74° F. The relative humidity was usually between 70 and 80. A summary of the number of days spent in each developmental stage and the total time from the egg to the adult stage is given in Table 1. These data were obtained from observations of nine lots of eggs from which approximately 3,600 adult *A. quadrimaculatus* were reared. It will be observed that the number of days from oviposition to the emergence of the adult mosquitoes average 21 days with a maximum of 27 and a minimum of 14 which is in fairly close agreement with the observations of Crowell who reported the period from egg to adult as varying from 19 to 27 days at air temperatures of about 75° F. A comparison with the data of Hurlbut is of interest. He reported a mean, maximum, and minimum number of days from egg to adult at con-

*This paper was presented at the annual meetings of the National Malaria Society in St. Louis, Missouri, 14 November 1944.

stant temperatures of about 74° F. as 16, 18, and 15 days, respectively, and at outdoor temperature averaging 74°F. as 21, 23, and 18 days, respectively. Thus, his mean developmental rate under outdoor conditions of varying temperatures was the same as under the insectary conditions of the present studies, while his rates at constant temperatures indoors were much more rapid. Huffaker (1944) has reported the time of development of *A. quadrimaculatus* from time of oviposition to adult at constant temperature of 76.5° F. as about 12 days. This corresponds fairly closely with the maximum rate of development (14 days) indicated by the present studies and with Hurlbut's rates of development at constant temperatures. Huffaker found that the differences between velocities of development at variable temperatures and those at comparable constant temperatures ranged from +13.4 to −2.7 per cent. This is of paramount importance with reference to production under natural conditions and may explain the variation in developmental rates observed by Hurlbut. As suggested by Huffaker, inequalities in competition for food and inherent differences between strains may also be sources of variation in the developmental rates.

TABLE 1.

Tabulation of Information on the Rate of Development of the Various Stages of *A. quadrimaculatus* at Water Temperatures of about 74° F.

Stage	No. Days After Oviposition Until Completion of Stage		Mean Duration of Stage in Days
	Minimum	Mean	
Egg		2	2
Instar I	4	6	5
Instar II	6	9	5
Instar III	9	13	5
Instar IV	12	19	8
Pupa	14	21	2
Total Days – Egg to Adult: Mean – 21; Max. – 27; Min. – 14.			

Observation under insectary conditions at Wilson Dam indicate that at constant water temperatures of about 74° F. the mean duration of the pupal period is 44 hours, with a minimum of 42 and a maximum of 52, the rates being almost identical for males and females. Hurlbut gave the mean, maximum, and minimum durations of the pupal period at constant temperatures of about 74° F. as 52, 55, and 49 hours, respectively. Huffaker found the length of the pupal period to be 46 hours at a constant temperature of 76.5° F.

Emergence And Longevity of Adults

During the past season, detailed observations were made on two separate colonies comprising a total of approximately 1,200 *A. quadrimaculatus* adults. The adults of the first colony emerged

on January 24 and 25 and those of the second colony emerged on July 28 and 29. In general, the emergence records showed about equal numbers of males and females. However, the peak of emergence of males always occurred sooner than for females. Thus, sex ratios must be determined from entire populations rather than from the emergence of a single day.

Information on the longevity of the adults from these two colonies is summarized in Fig. 1. The marked difference in the length of life of the males and females is apparent, 50% of the females surviving for 21 days while over 50% of the males had died by the end of the seventh day. The maximum length of life for a female was 62 days, while the last male died only 22 days after emergence. The maximum length of life of 62 days is considerably longer than the maximum span of 43 days previously recorded by Hurlbut (1941) at similar temperatures and humidities although Hinman and Hurlbut (1940) reported that hibernating females may survive for as long as 69 days.

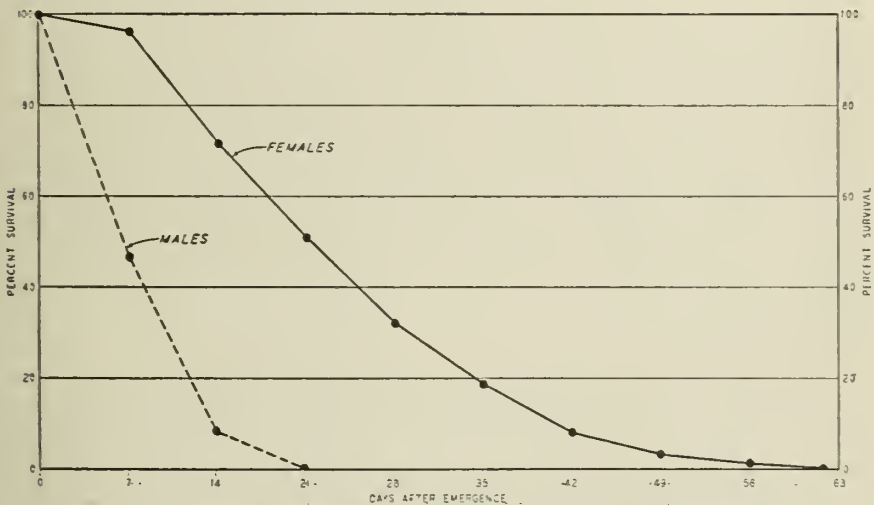

Fig. 1. Longevity of Male and Female Adult Anopheles quadrimaculatus under Insectary Conditions with Air Temperatures Between 76° and 80°F

Habits of Adults

In addition to the information on emergence and longevity obtained from the two colonies of *A. quadrimaculatus* previously mentioned, observations were made on the mating, ovipositing, and blood feeding habits of the adult mosquitoes. These observations are briefly discussed in the following paragraphs.

Mating

Mating was observed a number of times during the course of the experiments and occurred most frequently about 8 o'clock in the evening. Attachment took place during flight with both sexes moving very slowly and gradually descending to the floor of the cage. After remaining attached for 10 to 15 seconds, they gave a slight jerk and parted with the female flying off first and the male shortly after. During the periods when mating was observed, the colony was in a state of high activity which might be termed swarming. The cage in which the mating took place was approximately 24 inches long, 18 inches tall, and 10 inches wide.

The act of mating may apparently occur at any time after emergence. Virgin blood-fed females were admitted to a cage containing an abundance of males, and the females readily deposited fertile eggs on the third night following. Also, fertile ova were deposited by females which were placed with males before receiving a blood mean, then fed, and subsequently kept isolated until oviposition took place. Observations were made on the length of time a female would remain fertile after a single mating. A number of females deposited several batches of fertile eggs following one insemination, and one female deposited five successive fertile batches of eggs over a period of 17 days following a single insemination. Although a female may frequently mate a number of times, apparently one insemination is all that is necessary for her to remain fertile during her entire span of life. This might be expected from the much shorter length of life of the males which is indicated in Fig. 1. Under natural conditions, it appears that the females may mate soon after emergence and then migrate in search of blood meals and subsequently oviposit without further need for insemination.

Blood Feeding

Information on the blood feeding habits of *A. quadrimaculatus* adults was obtained by placing an arm in the cage for a 20-minute period at four-hour intervals during the life of the colony and counting the number of blood meals taken. During the course of the studies females were observed to take a blood meal as soon as 18 to 20 hours after emergence. In general, females never took more than one blood meal during a given day although in the first few days after emergence, the number of blood meals per female averaged as high as 1-½ indicating that some females had taken several blood meals during one day. In both colonies, the last surviving female took a blood meal on the last day before death. Frequently, adults were observed to die shortly after taking a blood meal, particularly during the later life of the colony. The average total number

of blood meals per female for the two colonies was 9.4. This means that the female with the maximum life span probably took 25 to 30 blood meals. Observations by Dr. R. L. Metcalf indicate the average amount of blood ingested by a female at a single feeding is about 0.003 cc. Frequently, females with partially digested blood meals were observed to feed again but, in general, they did not appear to take additional blood meals during the approximately 60-hour period between complete engorgement and oviposition. For both the winter and summer colonies the peak in blood feeding occurred 2 to 3 days after the emergence of the adults.

Observations were also made on the relation of light and time of day to blood feeding. A summary of 29 days of observations is given in Fig. 2. The observations were made in a cage placed next to the window of the insectary which faced west. The light was recorded as the amount reaching the cage through the window. The light conditions in the cage would probably approximate fairly closely those in a moderately open woods. It will be observed that under these conditions considerable blood feeding occurred during the daylight hours, but there was a sharp increase in the amount of blood feeding as soon as it became dark, and this increase continued through the night, reaching a maximum early in the morning, following which there was a rapid decline with the coming of daylight. The occurrence of the peak of blood feeding late at night might be associated with the fact that the peak of egg-laying occurs early in the evening; thus, any females which would not be occupied with oviposition early in the evening might turn to blood feeding later in the evening.

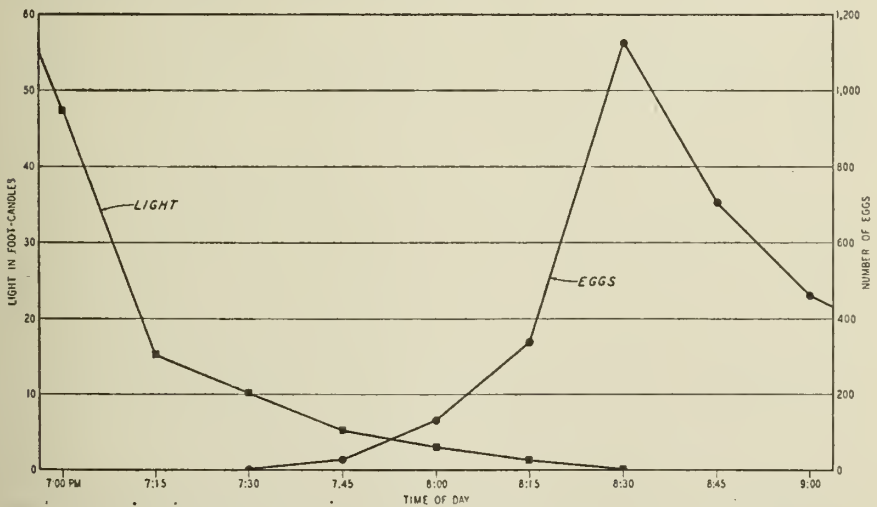

Fig. 2. Relation of Light and Time of Day to Blood Feeding and Oviposition of Anopheles quadrimaculatus Under Insectary Conditions at Air Temperatures of 76° to 80°F.

Oviposition

Oviposition was observed several times during the course of the experiments and usually proceeded as follows. The female rested on the side of the paper toweling at the edge of the water in the oviposition bowl with the hind legs in the air and the abdomen in a horizontal position. The pearly white eggs appeared one by one at the tip of the abdomen and each was released by a convulsive jerk at intervals of 2 to 3 seconds . A strong light directed on the insect during this time did not interrupt the procedure. By the end of 30 minutes, the eggs had turned to gray and had assumed their normal dark appearance by the end of 45 minutes.

Oviposition bowls lined with filter paper (Cromwell, 1940) were kept in the cage during the entire life of each experimental colony. The bowls were changed at each two hour interval and the eggs were counted. The first oviposition generally took place about 3 days after emergence and approximately 60 hours after the first blood meal. Thus, oviposition by a colony of *A. quadrimaculatus* occurs on the third night following the day on which they are given a blood meal. The peak of oviposition by a colony of adults emerging on a given day usually took place about a week after emergence.

Oviposition records were obtained from five *A. quadrimaculatus* females kept individually in small bobbinet cylinder cages 6 in. tall and 4 in. in diameter. The number of eggs per batch ranged from 194 to 263 with an average of 219 ±12. Some individuals oviposited as many as 9 to 12 times during their life span which would give a maximum egg production capacity per female of between 3,000 and 4,000 eggs. Detailed records on some 300 adult female *A quadrimaculatus* showed a total egg production during their life span of almost 200,000 eggs or approximately 660 eggs per female. An average of about 65 eggs was deposited for each blood meal taken.

A summary of observations on the relation of light and time of day to oviposition is presented in Fig. 2 These observations were made at two-hour intervals over a total period of 29 days. It will be observed that no eggs were deposited during the middle of the day, but oviposition began as soon as light readings approached zero and reached a maximum during the two-hour period immediately following total darkness. Thereafter, the oviposition dropped off sharply although some egg-laying took place during the remainder of the night until daylight the following morning. As previously pointed out, oviposition appears to be the dominant activity during the first part of the night and blood feeding the last part of the night.

In order to provide more detailed information on the relation of light to oviposition, observations were made at 15-minute intervals during the two-hour period immediately preceding and following darkness on eight different days. The results are summarized in Fig. 3. It will be observed that a few eggs were deposited when the amount of light dropped below 10 ft. candles; however, significant numbers of eggs were not deposited until the light had dropped almost to zero. The highest number of eggs was deposited in a very brief interval just at the time when total darkness occurred, and thereafter dropped off rapidly.

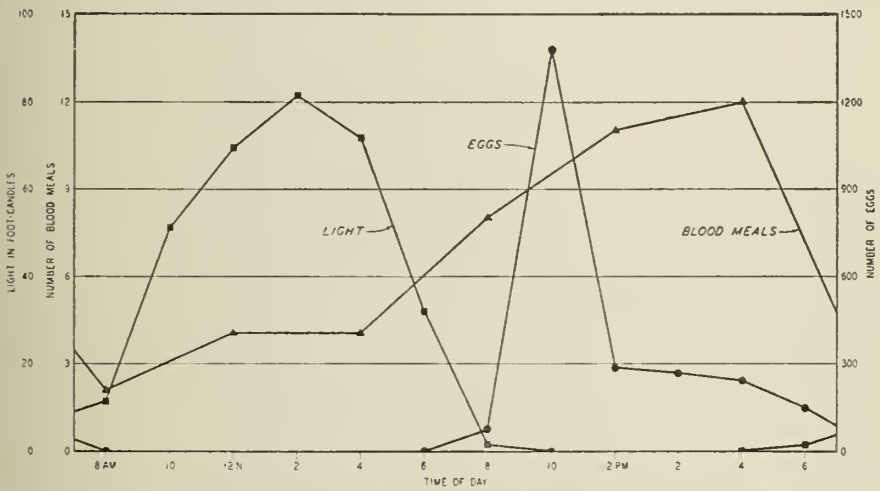

Fig. 3. Relation of Light to Oviposition of Anopheles quadrimaculatus Under Insectary Conditions at Air Temperatures of 76° to 80°F.

Summary

Life history studies of *Anopheles quadrimaculatus* were conducted in connection with the routine management of an insectary colony in the malaria control laboratories at Wilson Dam, Alabama. Information regarding the life cycle and habits of *A. quadrimaculatus* at relative humidities of 70 to 80, air temperatures of 76 to 80° F., and a water temperature of about 74° F. may be summarized as follows:

1. The mean number of days required from oviposition until emergence was 21 days with a minimum of 14 and a maximum of 27 days. The mean duration for each stadium from egg to pupa was two, five, five, five, eight, and two days, respectively. The mean duration of the pupal period in hours was 44, with a maximum of 52 and a minimum of 42.
2. The mean length of life of the female adult was 21 days and for the male, 7 days. The maximum length of life of a female was 62 days and of a male, 21 days.
3. Mating occurred as early as the first day of emergence and either before or after the first blood meal. Only one insemination was necessary for a female to continue to produce viable ova throughout her life span. Mating activity took place most frequently during the first few hours of darkness.

4. Females were observed to take blood meals as early as 18 to 20 hours after emergence. The total number of blood meals per female averaged 9.4.

   Some blood feeding occurred during the day but rapidly increased immediately after darkness occurred, reaching a peak near the end of the night.

5. The female deposited eggs while at rest on the water surface or on some object near the edge of the water. The eggs were released individually from the tip of the abdomen and were at first a pearly white, gradually turning to a glossy black in approximately 45 minutes. Oviposition occurred as soon as 80 hours after emergence and usually took place 60 hours after a blood meal. The average number of eggs deposited by one female was approximately 200 with a maximum of 300. No egg-laying occurred during the day but reached a peak immediately after the onset of darkness and then gradually diminished during the remainder of the night.

Acknowledgments

The writer is indebted to Miss Caroline E. Wilson for her able assistance which made possible the carrying out of continuous day and night observations on oviposition and blood feeding.

Acknowledgment is also made to Dr. A. D. Hess for his assistance and direction during the course of the studies.

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THE PHYSIOLOGY OF THE SALIVARY GLANDS OF *ANOPHELES QUADRIMACULATUS**

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Introduction

The literature on the salavary glands of mosquitoes is very meager, notwithstanding the importance of these organs in the transmission of disease and the infliction of irritating bites. Cornwall and Patton (1914) have studied the salivary glands of *Anopheles rossi* and *A. jamesi,* while York and Macfie (1924) have studied those of *A. maculipennis, Culex pipiens, Theobaldia annulata,* and *Aedes aegypti.* However, to the writer's knowledge there is no information of this sort available for American anophelines.

Methods

The mosquitoes used in this study were insectary reared. The salivary glands were carefully dissected in 0.85 per cent NaCl solution and treated as described. The effect of salivary gland emulsions on blood coagulation was measured by the standard capillary tube method (Kolmer Et al, 1931. A capillary tube about 1 mm. in diameter was filled with a mixture of blood and salivary gland emulsion, and small pieces were broken off at intervals and slowly separated. When the fibrin threads were seen to spread a distance of 5 mm. or more, coagulation was considered complete. All blood used was that of the writer unless otherwise specified.

Hydrogen ion concentrations in the salivary glands were determined by means of standard indicators using color comparison with mixtures of the same indicator and standard buffer solutions. The indicators used and their intervals of color change were as follows:

bromothymol blue	Yellow-Blue	6.0-7.6
bromocresol purple	Yellow-Purple	5.2-6.8
phenol red	Yellow-Red	6.4-8.0

Enzyme determinations were made by macerating salivary glands into a suspension in a 50:50 mixture of phosphate buffer, pH—6.8[1], with glycerol and a little alcoholic thymol. Two glands were used in each determination, being macerated in 10 mm^3 of saline. Substrates were made as follows: protease—2 per cent gelatin in water; amylase—2 per cent starch in water; lipase—an emulsion of pure olive oil with a trace of bromothymol blue indicator, ad-

[1]Equal portions of a solution of 9.078 g. KH_2PO_4 in 1 liter and 11.876 g. of $Na_2HPO_4.2H_20$ in 1 liter.

*This paper was presented at the annual meetings of the National Malaria Society in St. Louis, Missouri, 14 November 1944.

justed to a faint blue with dilute NaOH solution. Approximately 10mm³ of substrate and an equal amount of salivary gland emulsion were mixed, sealed in capillary tubes, and incubated for 72 hours at 37° C. Controls of substrate and saline were treated in the same manner.

Following the incubation, the gelatin substrate was tested for the presence of free amino acids by Folin's method using sodium B-naptho-quinonesulfonate and sodium carbonate and for changes in protein composition by the biuret test (Hawk and Bergeim, p. 427). The starch substrate was tested for the presence of reducing sugars by treatment with Benedict's reagent (Hawk and Bergeim, p. 846). The olive oil substrate was tested for the presence of fatty acids by observations on the change in pH as measured by the color of the bromothymol blue indicator.

Results

General observations on the morphology and physiology of the salivary glands

The salivary glands of *A. quadrimaculatus* females are very similar in appearance to those of *A. maculipennis* as figured by Patton and Evans (1929). Each gland consists of 3 lobes, 2 lateral acini, and a much shorter acinus. The lateral acini are approximately 900 microns long and about 70 to 80 microns wide. They consist of two distinct morphological divisions of about equal length. The proximal portion consists of large elongate cells bordering upon an intra-acinar duct, which is about 3 microns wide. These cells have indistinct nuclei. The distal portion consists of very distinctly nucleate cells having a granular appearance. The canal ends at about the point where the cell type changes, the duct opening into an elongate cavity, about 1/5 of the gland width, extending nearly to the blind end of the gland. Minute globular accessory chambers can occasionally be found opening into the ducts. The median acinus is much shorter than the others, being about 360 microns long. Its cells resemble those of the distal portions of the lateral acini but the cellular divisions are less distinct. The duct to the central acinus is very short and ends in a cavity about one-third of the gland width. The three ducts from the acini join into a duct having taenidial rings, resembling those of the tracheae. The ducts from each gland unite to form a common salivary channel which passes into the stomadeum near the proboscis.

The total volume of each gland, calculated from the measurements given above, is about $8 \times 10^{-3} mm^3$.

Chemical Properties of Salivary Glands

The salivary glands and their contents give a strong biuret reaction with copper sulfate solution and dilute alkali. The color

formed is a light pink, indicating a protein of low molecular weight or a polypeptide of more than 3 amino acid residues. (Gortner, p. 385).

The glands gave no evidence of lipid staining with Sudan III dye, nor were any doubly refractive lipids revealed upon examination with the polarizing microscope.

Microscopic examination by the fluorescence technique revealed a bright blue-violet fluorescence of the intra-acinar ducts and the salivary ducts. Such blue-violet fluorescence is characteristic of chitin, and indicates that the salivary glands, which are derivatives of the stomadeum, probably contain the chitinous intima which is characteristic of that portion of the insect alimentary tract.

The differing historical structures of the middle and lateral acini have led authors to speculate on differences in their functions. This has been confirmed by measurements of the hydrogen ion concentration of the various lobes. Measurements with indicators have led to the following results:

Indicator	*Median Acinus*	*Lateral Acinus*
Bromothymol blue	grass green to blue green pH=6.8-7.0	yellow to yellow green pH=6.0-6.2
Bromocresol purple	deep blue pH=6.8	bright green pH=6.0
Phenol red	yellow orange pH=7.0	yellow pH=<6.8

Enzymes in the Salivary Glands

Tests of salivary gland emulsions for the presence of protease, lipase, and amylase were all negative.

Lecithinase "A" is a toxic constituent of bee, scorpion and snake venoms. A test was made for its presence in the salivary glands of *Anopheles quadrimaculatus* females, using the method given by Sumner and Somers (1943). The salivary glands were dissected from 20 mature females and ground in a mortar with 1 cc. of egg yolk and 20 cc. of phosphate buffer of pH=7.1. The resultant solution gave no laking of human blood either before or after 24 hours incubation of 47-49° C. Thus, it is improbable that lecithinase "A" is present in the salivary secretions of this mosquito.

Properties of Agglutinin

From 10 to 15 seconds after the addition of a small amount of human blood to saline containing a crushed salivary gland, marked agglutination or clumping of red cells occurs. No agglutination, however, occurred with the glands of newly emerged females, the agglutinin apparently not being developed. This material appears to develop 8 to 12 hours after emergence. By heating mosquitoes at 50 to 55° C. for 5 to 10 minutes, the agglutinin was destroyed, and crushed salivary glands from these insects gave no agglutination

reaction. Mosquitoes that had been freshly fed were found to contain strongly agglutinated blood in their stomachs, but this could be restored to normal appearance by heating at 50 to 55° C. as could drops of blood agglutinated by the mosquito salivary glands.

To determine if the source of the agglutinin could be confined to a particular portion of the salivary gland, tests were performed in which only a single lobe of a complete gland was crushed in the saline drop. The procedure was rather difficult in that rupture of other parts of the gland during the operation frequently occurred as did discharge of glandular secretions through the free end of the salivary duct. These factors sometimes produced erratic results, but, in the majority of the more than 30 tests performed, very intense agglutination occurred with the contents of the median acinus, while the contents of the lateral acini gave no or only a very feeble agglutination.

To ascertain the maximum dilution at which agglutination would occur, successive dilutions of single salivary glands were made in 0.85 per cent NaCl solution. From a consideration of the total volume of the median acinus ($1.4 \times 10^{-3} mm^3$.) of a single gland as the maximum amount of agglutinin available, the following dilution values were obtained.

Dilution	*Agglutination*
1:5,000	10 to 15 seconds—strong
1:10,000	15 to 30 seconds—strong
1:20,000	1 to 2 minutes—strong
1:100,000	4 to 12 minutes—strong
1:1,000,000	22 minutes—weak

The action of the agglutinin on a variety of different animal bloods was studied. Strong agglutination occurred with the blood of mule, cow, pig, dog, rabbit, guinea pig, rat, and mouse; however, the blood of the chicken and of a turtle *(Chelydra serpentina)* gave no agglutination in repeated trials.

The four human blood types, A, B, AB, and O, were tried for agglutination by the salivary gland material, and all gave strong agglutination.

Properties of Anticoagulin

The salivary glands of the female mosquitoes contain a powerful anti-coagulin, which is active in dilutions of 1:10,000 for human blood. This in indicated by the results in table 1.

As seen from the table, there appears to be no significent difference in the amount of anticoagulin present before and after feeding. The anticoagulin is thermostable and is not affected by heating to 55° C. for 15 minutes. By crushing either the lateral or median acinus of a gland in saline and then applying the coagulation test,

TABLE 1.
Comparisons of Clotting Times of Normal Human Blood With Blood Plus Anticoagulin from Salivary Glands of *A. quadrimaculatus* Females.

	Material	*Clotting Time*
1.	(a) blood—10mm.	1 to 2 min.
	(b) blood—10mm + 1 crushed salivary gland (unfed mosquito)	15 min.
2.	(a) blood—10mm. in 10mm. saline	9 min.
	(b) blood—10mm. in 10mm. saline + 1 crushed salivary gland (unfed mosquito)	60 min.
3.	(a) blood—10mm. in 10mm. saline	5 min.
	(b) blood—10mm. in 10mm. saline + 1 crushed salivary gland (after feeding)	30 min.
4.	(a) blood—5mm. in 5mm. saline	6 min.
	(b) blood—5mm. in 5mm. saline + crushed salivary gland (after feeding)	60 min.
5.	(a) blood—5mm. in 5mm. saline	4 min.
	(b) blood—5mm. in 5mm. saline + 1 crushed salivary gland (1 day after feeding)	45 min.

it was found that extracts of either lobe were effective in inhibiting coagulation, but in the majority of the tests performed the anticoagulation properties of the median acinus were more pronounced than those of the lateral acinus. The anticoagulin is not present in newly emerged adults but appears to form sooner after emergence from the pupae than does the agglutinin.

Comparison with Other Mosquitoes

The salivary glands of a number of other mosquitoes were examined for the sake of comparison with *A. quadrimaculatus*. The glands of *A. punctipennis* and *A. crucians* contained both an agglutinin and an anticoagulin, but the other species examined, *Aedes aegypti, A. vexans, Culex salinarius, C. quinquefasciatus, C. restuans, and Psorophora discolor*, contained neither an agglutinin nor an anticoagulin for human blood.

Adult female *Aedes aegypti* and *Anopheles quadrimaculatus* were fed on human blood and dissected in saline 15 minutes after feeding. At this time, the blood in the stomach of the *A. aegypti* was coagulated and not agglutinated, while in the *A. quadrimaculatus* the blood was strongly agglutinated and not coagulated.

Influence of Salivary Gland Contents in Producing Symptoms of Bite

Salivary gland emulsions in saline solution, injected intradermally in the forearms of two subjects produced typical symptoms of mosquito bites. Within one or two minutes after the injection, a small hyperaemic wheal appeared at the site of the injection. This persisted for 24 to 48 hours as is characteristic for *A. quadrimaculatus* bites on these particular individuals. When the injection was

repeated using glands from mosquitoes that had been heated to 55° C. for 15 minutes, no symptoms occurred.

Salivary Glands of the Male of Anopheles quadrimaculatus

The salivary glands of the male are irregular in form, a condition which may be associated with their degeneracy. In the specimens examined each gland seemed to consist of a single lobe with one or more lateral diverticula of variable size. Nothing corresponding to the median acinus of the female gland was found. The male glands are about one-third of the dimensions of the female gland and have less than one-twentieth the volume. The cells of the male glands are less distinctly nucleated than those of the female glands. The intra-acinar ducts pass almost the entire length of the acini and, in contrast to the female glands, do not widen into cavities in the distal portions of the glands. This condition would seem to indicate a lesser degree of secretory activity of the male glands.

Emulsion of the crushed male glands when mixed with human blood gave no indication of the presence of agglutinin or anticoagulin factors.

Discussion

The conclusions of Cornwall and Patton (1914) and York and Macfie (1924) regarding the presence of agglutinin and anticoagulin factors in the salivary glands of *Anopheles maculipennis, A. rossi,* and *A. jamesi,* together with the results of the present study indicating the presence of these factors in *A. quadrimaculatus, A. punctipennis,* and *A. crucians,* offer strong evidence that these factors are widely distributed in the genus *Anopheles.* However, studies of other genera of mosquitoes have indicated that such factors are not present. It seems possible that the presence or absence of these materials may determine the physiological reaction of the host to the mosquito bite and may be correlated with the relative abilities of different mosquitoes to serve as vectors for various diseases.

The differing pH of the lateral and median lobes of the salivary glands and the presence of the agglutinin in the central acinus, but not in the lateral acini, indicate a diversity of function. The site of formation of the anticoagulin is somewhat in doubt, but it appears to be present in the median acinus in far larger amounts than in the lateral acini.

The fact that the anticoagulin and agglutinin are not present in the salivary glands of newly emerged adult female mosquitoes and do not become completely developed for at least 8 to 12 hours after emergence appears to correlate with the recent study of Keener (1945) who states that these mosquitoes do not seek a blood meal until 18 to 20 hours after emergence.

The fact that the salivary glands of the male of *A. quadrimaculatus* are much smaller than those of the female and do not contain the agglutinin and anticoagulin factors for blood appears to be entirely consistent with the food habits of the males which, at least, in the insectary colony maintained subsist purely on fruit juices and dextrose solution.

Summary and Conclusions

1. The salivary glands of *A. quadrimaculatus* and their contents appear to be largely protein in nature.
2. The intra-acinar ducts of the salivary glands show the characteristic blue-violet fluorescence typical of chitin.
3. Tests for the presence of amylase, protease, and lipase in the salivary glands were negative.
4. The median acinus of the salivary gland is considerably more basic (about pH 7) than the lateral lobes (about pH 6).
5. The salivary glands contain an anticoagulating agent for human and mammalian bloods which is thermostable and active at dilutions of more than 1:10,000.
6. The salivary glands contain a powerful agglutinin for human blood types A, B, O, and AB, and for mule, cow, pig, dog, rabbit, guinea pig, rat, and mouse blood. Chicken and turtle blood were not agglutinated. This material is thermolabile and active in dilutions of more than 1:1,000,000.
7. The agglutinin is apparently produced only in the median acinus of the gland which also appears to contain the most active anticoagulin.
8. Neither the agglutinin nor the anticoagulin is present in the salivary glands of newly emerged adult female mosquitoes.
9. The salivary glands of a number of species of pest mosquitoes were examined and were found to contain neither an agglutinin nor an anticoagulin for human blood.
10. Blood in the stomach of *A. quadrimaculatus* shortly after feeding was found to be uncoagulated and agglutinated, while blood in the stomach of *Aedes aegypti* was coagulated and non-agglutinated.
11. Emulsions of *A. quadrimaculatus* salivary glands in saline gave a typical "bite" reaction when injected into the human skin. Similar emulsions heated to 55 to 60° C. for several minutes gave no reaction upon injection.
12. The salivary glands of the male of *A. quadrimaculatus* are much smaller and apparently less active than those of the female. The male glands do not contain agglutinin and anticoagulin factors for blood.

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